DEVELOPMENT OF AN ANIMAL MODEL FOR THE EVALUATION OF

ORAL CONTROLLED RELEASE PREPARATIONS: STUDIES WITH INDOMETHACIN

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To the mind where Gateleaper flies.

DEVELOPMENT OF AN ANIMAL MODEL FOR THE EVALUATION OF ORAL CONTROLLED RELEASE PREPARATIONS: STUDIES WITH INDOMETHACIN.

Carlos Ramon Garcia

Two animal models for the evaluation of oral controlled release dosage forms under physiologically stabilized conditions have been developed. Unanesthetized restrained male rhesus monkeys with chronic vascular catheters and a plastic cannula surgically implanted in the stomach were utilized as the basic set-up. By radiological means it was established that the intestinal transit time in fasted, unanesthetized (restrained) rhesus monkeys was considerably faster than the corresponding value in man. In the models, passage to the large bowel was prevented and the small intestinal contents were collected at the terminal ileum and readministered at the upper-jejunum. For Model I, surgical techniques were developed for the implantation of plastic cannulae in different portions of the small intestine of the animal: upper-jejunum and terminal ileum. For Model II, an ileostomy was performed, the colon permanently closed and a plastic cannula implanted in the upper-jejunum of the monkey.

Radiological studies were conducted to determine if the extensive surgery performed on Model I and II preparations had affected the gastrointestinal transit time. The post-surgery values found for both models were not significantly different.

Models I and II allow the investigator to study the absorption of drugs from controlled release preparations in a gastrointestinal system anatomically and physiologically similar to the g.i. system

in humans. The models allow the direct administration of intact solid dosage forms into the stomach of the animal. The models allow the investigator to intravenously administer drug solutions and to sample the peripheral blood compartment frequently so that pharmacokinetic parameters of a drug can be determined. The models allow the controlled release dosage form to be in contact with the absorbing mucosa for a period of 8 to 12 hours. The models allow the investigator to run repeated studies on the same animal.

The models developed were tested utilizing experimental controlled release preparations of indomethacin (hard-gelatin capsules containing either coated granules of the drug, or drug embedded in a plastic polymer matrix).

Intravenous studies with indomethacin and oral studies with the drug in solution, in conventional (in-line) capsules and the controlled release preparations were conducted.

Pharmacokinetic parameters for indomethacin disposition in eight rhesus monkeys were obtained following the intravenous administration.

The Loo-Riegelman method was used to estimate the cumulative amount of drug absorbed from plasma concentration time data obtained after i.v. and oral administration of indomethacin to monkeys. In six monkeys, the extent of the enterohepatic recycling for up to 24 hours averaged 49.8% (S.D.=39.5). The latter estimate agrees closely with the 50% reported in man.

Our studies show that indomethacin is rapidly absorbed when given orally as a solution. Estimates of total area under the plasma concentration time curve (AUC) corrected to the administered dose and body

weight, percent of dose "absorbed" (Loo-Riegelman method), and relative bioavailability for the dosage forms under study were obtained. The enterohepatic recycling of indomethacin proved to be a complicating factor in the rigorous evaluation of the dosage forms under study. Nevertheless, a relative estimate of the in vivo performance of the various formulations in the same monkey was obtained by means of the ratio of total corrected AUC of the dosage form being evaluated to the total corrected AUC of the in-line preparation (relative bioavailability). Additionally, rate plots for some of the dosage forms under study were constructed from the cumulative amount of drug "absorbed" data.

Analysis of all data obtained lead to the conclusion that from the five experimental controlled release dosage forms of indomethacin tested, only two preparations showed in vitro and in vivo availability characteristics that justify their further testing in human subjects.

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I. INTRODUCTION

Controlled release preparations are those specially designed dosage forms in which the rate of release of the active ingredient(s) is such so as to immediately attain therapeutic concentrations of the drug, and/or metabolite(s), at target sites and to maintain these levels for longer periods of time, providing in such a way a clinical advantage over the conventional formulations of the same chemical entity (1).

Since the Food and Drug Administration (FDA) considers oral controlled release preparations to be new drugs, safety, efficacy and evidence of controlled release must be included in any New Drug Application (NDA). Bioavailability studies are more sensitive and accurate than clinical trials in providing evidence of controlled release. However, it is the FDA's principle that only necessary human research should be carried out. Therefore, bioavailability testing shall not be conducted in humans if an appropriate animal model exists and correlation of results in animals and humans has been satisfactorily demonstrated. Previous attempts have been made to evaluate controlled release preparations in animal models (2-4). Unfortunately, the short gastrointestinal transit time of the animals utilized has limited the observation period (3, 4). Thus, at the present time there is no satisfactory animal model which would allow the dosage form designer to evaluate an oral controlled release formulation, without immediately testing the dosage form in man.

The purpose of this research project is to develop an animal model for the comparative evaluation of oral controlled release products under physiologically stabilized conditions. It was postulated that the model should comply with the following requirements:

1. The gastrointestinal system of the animal should be similar anatomically and physiologically to the gastrointestinal system in humans.

2. The model should allow the investigator to orally administer intact dosage forms without using anesthesia or causing undue stress to the animal. The use of anesthesia during bioavailability studies is ruled out since it is known that there are blood flow and gastrointestinal motility alterations associated with anesthesia (5-8).

3. The model should enable the dosage form to be in contact with the absorbing mucosa for a period of at least 8-12 hours.

4. The model should allow the investigator to sample blood frequently and to run repeated studies on the same animal.

5. The model should be available for long periods of time during the course of each experiment (for example, up to 24 hours) in an un-anesthetized state.

In the present work, the next chapter represents a literature survey of pertinent and related research. Chapters III to V deal with the experimental, results and discussion parts, respectively; the last chapter summarizes our findings.

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II. LITERATURE SURVEY

A. Controlled Release Preparations

1. Introduction.

In order to have effective drug treatment, a therapeutic concentration of a suitable drug at the site of action is required. Once a specific drug is selected then the objective is to administer it correctly. Considerations of the correct route of administration and dosage form are usually based on data derived from experiments in animals (pharmacologic, toxicologic, metabolic and pharmacokinetic studies); nevertheless, the way the drug acts and interacts with the human body must be studied (phase 1 testing) before a suggested dosage schedule may be proposed for man. The dosage form, i.e., the form of the completed drug product, may be thought of as a drug delivery system. Almost any change made in the formulation can affect the rate at which the drug appears in the systemic circulation and also the ratio of the amount of drug entering the circulation to the dose originally given in the dosage form. In many cases, for drugs given orally, the rate at which a drug dissolves from its dosage form in the human gastrointestinal (g.i.) tract controls the rate of absorption. After entering the vascular system drugs are distributed into various parts of the body and at the same time are eliminated; in general, distribution takes place more rapidly than elimination. In some instances, drugs are administered only once; usually, however, they are repetitively administered to maintain a constant concentration of the active compound in the blood or tissues, seeking to obtain a uniform response. By adjustment of the dose and the dosage interval, optimum control is

attained. The ideal dosage regimen of a drug is that by which therapeutic levels of the drug at the site of action are immediately attained and maintained for the desired duration of the treatment. This ideal regimen is best accomplished by a constant intravenous infusion of the drug into the body, a procedure which in most cases is impractical except for hospitalized patients.

The goal of maintaining the ideal dosage regimen coupled with attempts to optimize conventional drug delivery systems (so as to maximize availability with a minimum amount of drug) has led to the development of dosage forms consisting of a protected supply of drug from which the drug is released at a controlled rate over a long period of time. This approach to drug delivery systems is based on the assumption that repeated application in small increments of the total dose of drug will approximate a constant infusion and produce the desired effect, that is, achieve the ideal dosage regimen. Products formulated using this approach have been described as sustained release, prolonged release, depot, timed release, delayed action, repository, sustained action, extended action, gradual release, retarded release, etc., thereby attempting to describe their mechanism of drug liberation and/or effect (1, 2). The concept of using a controlled release dosage form has been applied to a great variety of drugs including among others: pacemaker drugs, anesthetics, antimalarial and antischistosomal agents, atropine and histamine, and a variety of steroids for fertility control (3).

2. Rationale for Controlled Release Preparations.

The rationale for development and use of controlled drug release dosage forms may include one or more of the following:

a. To decrease toxicity and occurrence of adverse reactions by control of the level of the drug and/or metabolites in the blood and at depot sites.

b. To better drug utilization by enabling a smaller drug dose in a controlled release form to produce the same clinical effect as a larger dose in a conventional dosage form.

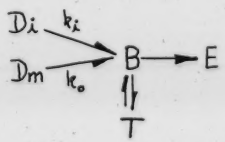
c. To control the rate and site of release of a drug that acts locally so that the drug is released where the activity is needed rather than at other sites where it may cause adverse reactions.

d. To provide more uniform blood concentrations and/or provide a more predictable drug delivery system.

e. To provide greater patient convenience and/or better patient compliance by significantly prolonging the interval between administration.

3. Formulation of Controlled Release Products.

The simplest model utilized for the consideration of theories governing the design of an oral controlled release dosage form, is one similar to an i.v. infusion with an initial rapid i.v. injection as a "loading dose." For controlled release preparations the difference is that both the initial and maintenance dose have an absorption step before entering the blood (4). Thus, the model may be written:



where D_i is the initial dose; D_m is the maintenance dose; and B, T and E represent the blood, tissues and elimination, respectively. It is

assumed that D_i is rapidly absorbed after oral administration following a first order process characterized by the absorption rate constant k_1. The zero order release from D_m is rate-determining for the maintenance portion of the dose and absorption of the maintenance dose may be characterized by the zero order release constant k_0. If the controlled release dosage form is to control the absorption, then the dosage form must first limit the dissolution rate; this is accomplished by the design of some physical barrier that will prevent and control the contact of the drug with the dissolving fluids. There are many methods by which the physical barrier may be built into the oral dosage form including the use of coatings, embedding drug in a wax-fat matrix, incorporating drug into a porous plastic base, microencapsulation and binding to ion-exchange resins (5). Commonly used structures for these kinds of preparations are: slow-erosion cores with an initial fast release dose, erosion cores only, coated pellets in capsules, pellets in tablets, resin-polymer beads, microencapsulates, inert plastic matrices, hydrophilic matrices, soft gelatin capsules, etc. The characteristics of these structures have been extensively discussed (1, 2). A number of investigators have applied rigorous pharmacokinetic principles to the design of controlled release dosage forms,(6-13) Equations, based on the release characteristics of hypothetical dosage forms, describing the plasma level-time course of the drug are relatively simple to develop. However, formulation of controlled release preparations which will yield these ideal plasma level-time profiles appears to be an exceedingly difficult problem. Due to the technical problem of producing a dosage form

which will release drug *in vivo* at a predetermined and consistent rate, it becomes difficult to discuss the pharmacokinetics of controlled release dosing in realistic rather than hypothetical terms (4).

4. Drugs Suitable for Controlled Release.

In considering the desirability of producing controlled release dosage forms, several factors should be taken into account: the biologic half-life of the drug, its general degree of absorbability, the gastrointestinal site of absorption, the conventional dosage regimen, the frequency and type of toxicity encountered, the relation of peak versus steady state levels in efficacy and toxicity, the minimum effective concentration and the minimum toxic concentration (4, 14). There is probably no rational reason for formulating a controlled release preparation for peroral use of a drug having a biological half-life, $t_{1/2}$, of 8 or more hrs. Drugs with a $t_{1/2}$ between 4 and 6 hrs can usually be easily incorporated in such formulations, but active ingredients having a $t_{1/2}$ of 1 hr or less are difficult to formulate into this type of dosage form if their usual single dose is high, i.e., more than 50 mg (1). It is important to know whether the prolonged release drug candidate is absorbed from all regions of the g.i. tract. Even if the drug is constantly released *in vitro* but is not absorbed from deeper parts of the intestines, a prolonged release preparation would be ineffective.

5. FDA Regulations for Controlled Release of Drugs.

Controlled release formulations normally contain a larger amount of drug than the single doses usually administered. There is a possibility

of unsafe overdosage if such products are improperly made and the active ingredients are released at one time or over to short a time interval. Thus, in the context of current FDA regulations, any such dosage form that contains per dosage unit a quantity of active drug ingredient(s) which is not generally recognized as safe for administration as a single dose, is regarded as a new drug (15). The requirement for the submission and approval of a full NDA for these products has been set out in the regulations (16); such a NDA must contain clinical evidence of safety and effectiveness, as well as data supporting the labelling claim of controlled release over a given time interval. The Drug Amendments of 1962 (17) explained that all claims for effectiveness must be supported by "substantial evidence," defining such "substantial evidence" in terms of adequate and well-controlled studies by experts, as distinguished from anecdotal evidence by individual practitioners.

In 1975 (18) the FDA Commissioner issued a proposed regulation on bioavailability requirements for human prescription drugs. Bioequivalence testing must be conducted using the most accurate and sensitive method available. A clinical trial to establish the safety and effectiveness of a drug product is the least accurate and sensitive of the methods set out in 320.2 (b) (2),(18), and is only adequate when other methods are not available. Section 320.2 (d) (4) of the proposed regulations provides that the guiding principle in bioavailability testing is that no unnecessary human research should be done. Thus, "bioavailability testing shall not be conducted in humans if an appropriate animal model exists and correlation of results in animals and

human has been satisfactorily demonstrated" (18). Cabana (14) has listed the requirements which should be met to demonstrate safety and efficacy for controlled release products. He considers two different classes of drugs:

a. Drugs which have been published in the Federal Register as safe and effective in conventional dosage forms.

i. Controlled clinical studies may be required to demonstrate safety and efficacy.

ii. Bioavailability data are required and may be acceptable in lieu of clinical trials, if it can be shown that the blood levels and/or urinary excretion rates are comparable to multiple doses of the appropriate conventional dosage formulations.

In such a case, the labelling must clearly state the recommended dosing regimen and must be identical with that of the standard formulation in terms of effectiveness and side effects. Any claims of clinical advantage must be substantiated by appropriate well controlled clinical studies.

b. Drugs which have been published in the Federal Register as safe and effective in a controlled release form.

i. Blood level and/or urinary excretion rates are required and acceptable when compared to the reference product.

ii. The labelling must be identical to the reference standard with regard to effectiveness and side effects. Without appropriate clinical trials the labelling cannot be changed with regard to clinical effectiveness or (decrease) in side effects.

iii. Demonstration of the controlled release nature.

B. Evaluation of Controlled Release Dosage Forms

1. *In Vitro* Tests.

Proper control of manufacturing processes and preliminary testing of sustaining methods and systems is accomplished by *in vitro* methods.

In vitro dissolution rate testing is an important tool in the design, evaluation and control of sustained release dosage forms. There is no "standard" dissolution rate method for this kind of preparation. The methods described in the literature (19, 20) vary according to the drug, "sustaining" materials and dosage form tested. Commonly employed methods include the use of simulated gastric and intestinal fluids at a temperature of 37°C, the use of a mechanical device to agitate the dissolving fluid and the product at a fixed stirring rate, and the use of a sieve to retain the disintegrated particles of the dosage form. The rate of disappearance of drug from the product, or appearance in the dissolution fluid, is measured as a function of time (21). The usual variations in these methods are: the sampling intervals, the fluid composition, the agitator and the size of the sieve utilized (22-25). Among the most commonly used techniques are that of Stoll-Herschberg (22) and of Sonder and Ellenbogen (25); both devices have been shown to produce data satisfactorily related to *in vivo* effects (21). Automated devices and radioactive techniques have also been reported (26, 27). The advantages and disadvantages of several of the dissolution rate procedures have been analyzed (28). It is apparent that although many *in vitro* testing methods are available, a completely general one, useful for all kinds of controlled release preparations is not available. A more detailed

discussion of *in vitro* testing and *in vitro-in vivo* correlations may be found in the review article of W. Barr (29).

2. *In Vivo* Evaluation.

a. Clinical trials.

Literature reviews pointing out the marked differences between the methods utilized for the *in vivo* evaluation of oral controlled release products have been published (30, 31). Ideally, such methods should include quantitative measurements of the drug activity or drug concentrations following the administration of the controlled release preparation as well as for the drug in solution or from fast release tablets for comparison purposes. However, in many instances it is not possible to obtain quantitative measurements either because of the low concentrations of drug in the body, or the lack of specificity of the assay method used. In such cases, a carefully conducted and controlled clinical trial would be appropriate. However, only a few of the reported studies satisfy the minimal criteria of scientific acceptability. Campbell et al. (31) and Campbell and Nelson (32) point out that an enormous number of clinical trials involving controlled release preparations consist of subjective measurements in non-controlled small groups of patients. Such studies do not justify the conclusion that the formulations tested had controlled release properties.

There is no doubt that the use of inadequate tests explains the presence on the market of preparations claiming controlled release which do not exhibit this property when evaluated by critical methods (21).

Of real concern in all clinical evaluations is the variability of natural processes within and between humans. Sufficient numbers of

patients must be investigated in single dose studies to provide an adequate cross-section of the important biological functions of the drug-taking population, and a similarly selected number of patients must be used in controlled release dosage form availability studies (21).

In vivo evaluation requires that the controlled release product should be compared with the same amount of drug in an immediate release form, as well as with more frequent smaller doses of the immediate release form so that the same amount of total drug for all three dosing schedules is administered. Placebos are only occasionally necessary. The requirements of blinding, systematized randomization, crossover in the same subjects, coupled with the complex dosing schedules required, create extremely involved design and analysis problems in carrying out these studies. Also, with multiple ingredients, a factorial design must often be superimposed to demonstrate additive effects, synergism, or antagonism (33). A large quantity of clinical work has been done to evaluate the performance of controlled release preparations in humans; however, only a small fraction of these results has been published. Modell and Houde (34) have discussed some of the factors influencing the clinical evaluation of drugs, making special reference to the double-blind technique.

b. Animal models.

Small animals such as mice, rats and guinea pigs cannot be used to study intact solid dosage forms. The use of larger animals, such as the dog, pig or monkey for routine testing of dosage forms would have obvious advantages relative to increased human safety and convenience in large crossover studies. The use of these larger animals may provide

a useful secondary standard for routine quality control of some drugs to indicate when human testing is required. They may also be useful in the development or screening of potential dosage forms. The miniature pig has found increasing favor as a research animal in bioavailability studies since it is comparable to man in many physiological functions. However, even though the size of the animal has been reduced, it still reaches about 100 kg, presenting handling problems. In addition, the animal is continually growing, which may present problems in long-term crossover studies (29).

Although the screening of controlled release dosage forms utilizing animal models is a commonly used step in the development of such preparations, very few reports documenting their application have appeared in literature.

The dog has been frequently used as an animal model for the evaluation of controlled release dosage forms. However, reduced bioavailability of sustained release tablets tested has been attributed to the faster g.i. transit time found in dog as compared to man. Cressman and Sumner (35) studied the plasma-level profiles and sustained release properties of aminorex fumarate tablets in beagle dogs and compared results with those obtained in human subjects. Although the sustained release tablets prolonged the absorption period of aminorex in both man and dog, the rates of absorption differed. Moreover, the data indicated that the dog only absorbed 70-80% of the administered dose of aminorex from the controlled release tablets. Human subjects absorbed 100% of the administered drug. A subsequent experiment with non-disintegrating controlled release tablets provided more information. In two of the

four dogs studied, the tablet was expelled in the feces in the 6th hour. In the two remaining dogs, the tablets were found in the 8-12 hr fecal sample. Such results suggest that the dosage form may traverse the gut faster in dogs than in man. Hetch et al. (36) have reported a similar case when testing oral sustained release dosage forms of sodium ^{131}I-o-iodohippurate as the test substance in ambulatory dogs.

Other parameters rather than blood levels, excretion rates and bioavailability of drugs have been utilized in the evaluation of oral controlled release preparations. For example, Shenoy et al. (37) report the use of acute toxicity as a method of assessing sustained release preparations. In their study, the lethal effect of drugs in several controlled release preparations was compared with that of the drug administered in solution or as a powder. For this purpose, toxic doses of amphetamine as pelleted preparations and as resinates were administered to rats weighing about 100 g. The procedure of surgical insertion of the dosage form directly into the stomach of the rat was found to overcome satisfactorily the difficulty of dosing whole capsules (or tablets) or pellets in capsules to animals of such a small size. However, anesthesia was utilized. Under these conditions, alterations of physiological factors such as blood flow and g.i. motility may influence the biological performance of the controlled dosage forms tested.

Up to the present time, no adequate animal model for the comparative evaluation of oral controlled release preparations under physiologically stabilized conditions has been reported. The need and usefulness of such a model served as the rationale for the present work.

C. Controlled Release and Gastrointestinal Absorption

1. Dissolution of the Drug.

In order for a drug to be transferred from the lumen of the g.i. tract to the general circulation, a drug must first be solubilized in the fluids at the g.i. absorption site.

For oral solid controlled release preparations drug bioavailability is usually rate-limited by the dissolution of the drug into the g.i. fluids. The rate of the dissolution process may be influenced by the physical properties of the drug itself, the dosage form and by environmental factors at the site of absorption. The dissolution process can be explained in terms of the simplified equation based on a diffusion layer model developed by Nernst and Brunner, as discussed by Benet (38), and Hoener and Benet (39):

$$\frac{dQ}{dt} = \frac{D}{h} \cdot S\,(C_s - C_g). \qquad \text{Eq. 1}$$

where Q = amount of drug dissolved

t = time

D = diffusion coefficient of the drug in the solubilizing fluids of the g.i. tract

S = effective surface area of the drug particles

h = thickness of a stationary layer of solvent around the drug particle

C_s = saturation solubility of the drug in the stationary layer, h

C_g = concentration of drug in the bulk fluids of the g.i. tract.

This equation was derived under the following assumptions: the drug

dissolves uniformly from all surfaces of the particles, the particles are spherically shaped and all of the same size, h is constant and both h and C_s are independent of particle size. Furthermore, for controlled release preparations, C_g is assumed to be very low and constant since in the ideal case, absorption is assumed to occur at a much faster rate than the rate at which the drug is being released from the dosage form.

It is apparent from Eq. 1 that the rate of availability is proportional to the solubility of the drug in the dissolving medium. Thus, the rate may be decreased so as to prolong absorption by decreasing the solubility of the active compound.

The solubility of the drug can be decreased by changing the drug molecule from a salt form into a non-ionized moiety, varying the counter ion of the salt form, changing the crystal form of the drug molecule, or forming a less soluble complex of the drug with a pharmacologically inert substance.

The greater the particle size the smaller the surface area for a given amount of drug. Thus, equation 1 predicts that dissolution rate will decrease as particle size increases. In vitro and in vivo demonstrations of the importance of the effective surface area of drug particles upon dissolution rate have been documented for phenacetin, griseofulvin, nitrofurantoin and sulfadiazine (38,39).

In a preceding section of this chapter the different methods utilized in the formulation of controlled release products have been mentioned. A physical barrier designed to prevent and control the contact of the drug with the g.i. fluids of dissolution is one of the most common

methods utilized. Ritschel (1) has listed diffusion of the drug molecule through the physical barrier, leaching of the drug from the barrier and erosion and/or dissolution of the barrier among the most common mechanisms of drug release from controlled release dosage forms.

Physiological (environmental) factors such as the body temperature, the pH, viscosity, volume and composition of the g.i. fluids, and the g.i. motility also influence the rate of dissolution of a drug.

2. Absorption of the Drug.

Factors influencing the rate of absorption may be related to the simplified form of Ficks' law as described by Benet (38):

$$\frac{dQ_b}{dt} = D_m A_m R_{m/s} (C_g - C_b) / \Delta X_m \qquad \text{Eq. 2}$$

where when applied to gastrointestinal absorption: Q_b is the amount of drug in the blood or serosal solution at any time, t; D_m is the effective diffusivity of the drug in the intestinal membrane; A_m is the area of membrane available for free diffusion; $R_{m/s}$ is the partition coefficient between membrane and solvent; ΔX_m is the thickness of the membrane; C_g, concentration of drug in the gut or mucosal solution at any time, t and C_b, concentration of drug in the blood or serosal solution at any time, t. The terms D_m and $R_{m/s}$ depend on the relative lipid-water solubility of the drug, the drug dissociation constant, and hydronium ion concentration at both sides of the membrane. A_m and ΔX_m are determined by the section of the g.i. tract where absorption occurs. The drug concentration, C_g, at the lumen of the g.i. tract is a function of factors such as stirring, temperature, micellar or complex formation, metabolism at the g.i. wall, chemical (pH) and enzymatic degradation. C_b depends on blood flow to the absorption

site, the volume of drug distribution, protein binding, drug metabolism and excretion. Detailed discussions of these factors may be found in the literature (38, 40, 41).

Among the physiologic factors that may influence the bioavailability of a drug from an oral controlled release dosage form are: gastric emptying time, intestinal motility, surface area and specific absorption sites in the g.i. tract, blood flow to the absorption site, g.i. secretions, metabolism at the membrane level and the first pass effect.

The human gastric emptying time may vary from 0 to 12 hours (42). Oral controlled release preparations can reduce the hydrolysis that some drugs may undergo in the acidic medium of the stomach. A prolonged gastric emptying time may minimize this benefit (19), or at least slow the access of the drug to its main site of absorption, usually the small intestine. Gastric emptying and factors affecting the time of stomach residence have been reviewed (38-42). Factors such as physical activity, emotional state, position of the body, presence or absence of food, volume and composition of a meal, other drugs, etc. are known to alter the stomach emptying time. For instance, it can be anticipated that taking a drug shortly before, after or with a meal may delay the rate of drug availability as a function of decreased emptying rate. Thus, the timing of meals relative to the timing of the oral dosing of a drug can influence the rate and possibly the extent of drug availability. Both decreased rate and extent of bioavailability of a capsule product of dicloxacillin (43, 44) and decreased rate and enhanced extent of bioavailability of tablets and capsules of nitrofurantoin (45), when taken with meals, have been documented.

The intestinal motility may influence the intestinal transit time of ingested substances as well as providing some stirring action to aid in the dissolution process. Degree of physical activity, age, disease state and emotional condition of a patient may increase or decrease intestinal motility. The effect that diarrhea may have on the absorption of drug from a controlled release preparation, where the intestinal transit time is reduced to a few hours, is obvious. On the other hand, constipation or drug-induced hypomotility, i.e., as a result of anticholinergic drugs, can also produce an irregular absorption time course from oral controlled release preparations (46).

The g.i. motility as reflected by the g.i. transit time is a biologic variable of particular relevance for oral controlled release preparations. It should even be considered an important experimental variable when evaluations of oral controlled release products are to be performed on animal models. In this work we will define the term gastrointestinal transit time as the time required for the passage of an administered substance from the stomach to the cecum.

The gastrointestinal transit time can be measured with several techniques. The location of a column of barium sulfate can be observed by radiological studies; transit time can also be measured by the appearance time in the stool of detectable materials, including powdered charcoal, indigo carmin, and glass beads. The beads or pellets may be labeled with a detectable nonabsorbed isotope, such as radiochromium. The time of transit has also been measured using an ingested telemetering capsule (47). Clinically, it may be assessed by the ingestion of a barium sulfate suspension and monitoring of its passage by radiological means. The average transit time with

non-flocculating barium preparations does not seem to be significantly different from that found with barium water suspensions. When any of the barium preparations are given after an overnight fast, the normally functioning human stomach is empty in 1 to 2 hours. However, about 4 hours are required for an ordinary mixed solid and liquid meal to leave the stomach (48). An average of 2 to 4 hours after ingestion has been reported for a water-barium suspension to reach the cecum (49). However, in many cases without evidence of intestinal disease, the radiopaque material does not reach the cecum in 4 hours. Golden (50) quotes the study of Marina-Fiol (1943), where the transit time in 49 normal individuals was investigated. After administration of 100 g of barium sulfate in water, the time at which the radiopaque material began to enter the cecum was recorded as follows:

Transit time (hr)	2.5-3	3-3.5	3.5-4	4-4.5	4.5-5	5-6
Frequency (%)	14	28	36	12	6	2

Lonnerblad (51), after oral administration of 200 ml of a 2:1 barium suspension in water to 8 hour-fasted young adults (ages 18-25), found a mean transit time of 3 hours with a range of from 0.5 to 9 hours.

Lockard et al. (52), studying ileal motility in monkeys report results which tend to indicate that ileal motility is a direct function of ingestion and therefore of time of feeding and quantity of food. Moreover, if these parameters are held constant, the daily ileum motility patterns are very regular for individual rhesus monkeys. Distal ileum activity tended to peak and then wane approximately at 6 and 12 hours, respectively, from start of feeding and peak again around 18 hours every second to third day. The data seems to be

consistent with that from human radiographic studies quoted by Hunt (53) showing the shadow of the cecum from approximately 4 to 9 hours after a meal.

When a drug is absorbed at specific sites of the g.i. tract by an active process, the time of contact of the drug with the absorption site is usually short. Thus, the formulation of such a drug as a controlled release dosage form might easily result in low drug bioavailability. Examples of drugs absorbed by an active process have been reviewed previously (42). Some of these substances, vitamin B_{12} and thiamine, have been formulated in controlled release preparations with the expected results, that is, low drug bioavailability (40).

Drugs that have crossed the gastrointestinal membrane are primarily removed as a function of blood flow. A decreased blood flow may decrease the rate of removal of passively absorbed drugs (54). Decreased flow could possibly also interfere with active transport systems due to the reduction of the supply of oxygen to the tissues. The absorption of highly permeable compounds such as very lipid-soluble or pore diffusible substances should be flow limited. Conversely, the absorption rate of drugs characterized by low membrane permeability may be independent of blood flow (39). Results from animal studies on the influence of blood flow on g.i. absorption of drugs (55,56) are in agreement with the predictions based on theoretical considerations. Therefore, it becomes clear that changes in intestinal blood flow might influence the absorption rate of drugs in several ways (38). Blood flow may be affected, among other causes, by physical activity, emotional and disease states and by drugs (39). For instance, patients in heart failure would

generally be expected to have a decreased cardiac output and therefore, a decreased splanchnic blood flow. In addition, redistribution of cardiac output during cardiac failure may lead to splanchnic vasoconstriction in patients (57). The decrease of intestinal blood flow during anesthesia in man and animal species has been well documented (58-61). For controlled release preparations, the effectiveness of the initial dose may depend on an adequate blood flow at the absorption site, but blood flow should have little effect on the prolonged release dose.

The acid medium in the stomach and enzymatic activity in the g.i. tract may cause the degradation of some substances before they can be absorbed. For this reason, it has recently been suggested that an immediate release preparation of aspirin may produce greater acetylsalicylic acid bioavailability than an equivalent dose of aspirin in a controlled release product (62). The metabolism of drugs by the intestinal microflora and its implications has been discussed (63,64). The bacterial enzymes, mainly β-glucuronidase, are responsible for important drug biotransformations occurring in the gut. Concentrations of anaerobic organisms increase to 10^7 to 10^9 per ml in the distal ileum; such concentrations are similar to those found in the colon and in feces (65). The hydrolysis of glucuronides in the intestine occurs mainly in the lower part of the intestine (66). In patients with an ileostomy, the ileal effluent contains high concentrations of bacteria, 10^5 to 10^8 per ml which are capable of bile salt deconjugation (67).

Mucin secreted by the g.i. epithelium may affect absorption. In addition to increasing the viscosity at the site of absorption, mucin

might form complexes with certain compounds as has been shown for streptomycin and some quaternary ammonium salts (40). The effect of mucin on the performance of oral controlled release products has not been studied but it is probable that it will be of little importance under normal circumstances. Similar comments can be made about the biliary salts secreted into the intestine. Their role in the dissolution of certain lipid controlled release dosage forms has not been established, but it seems logical that such effect would be towards a faster drug release.

Drug absorbed from the stomach and the intestine must first pass through the liver before reaching the sampleable circulation. Thus, if a drug is metabolized in the liver or excreted into the bile, some of the active drug absorbed from the g.i. tract will be inactivated before the drug can reach the systemic circulation and be distributed to its sites of action. If the metabolizing or biliary excreting capacity of the liver is great, the effect on the extent of availability will be substantial (68). Such substantial hepatic first pass effect has been measured for many drugs. Thus, the available fraction of an oral dose appearing in the sampleable circulation will be governed by the extent of drug absorbed from the g.i. tract, by the fraction metabolized in the gut membranes and by the fraction metabolized and/or excreted into bile following passage through the liver. When the hepatic clearance for a drug, i.e., lidocaine, propranolol, propoxyphene, salicylamide (68), is large relative to the hepatic blood flow, the extent of availability for this drug will be low when it is given by a route which yields first pass effects. As Benet (68) points out

"...the decrease in availability is only a function of the anatomical site from which absorption takes place and no amount of dosage form redesign can improve the availability." When the first pass effect is assumed to follow first order kinetics, the hepatic extraction is independent of the rate of drug availability. That is, "no matter when a drug molecule is absorbed from the g.i. tract and at whatever dose administered, the hepatic extraction and the extent of availability for that drug will remain constant." However, for saturable hepatic enzyme systems the hepatic extraction would vary depending on the concentration of drug in the hepatic portal vein. Similarly, metabolism in the g.i. membranes could also be saturated or not, depending on the dose and the rate of absorption. Under these conditions the area under the curve can no longer be used to determine the extent of drug availability. Salicylamide appears to be a drug where the first pass extraction may be dose dependent. Benet (38) has interpreted data for para-aminobenzoic acid (PABA) as reflecting a case where the rate of drug absorption modified by changes in stomach emptying, causes changes in the extent of drug availability due to saturation of first pass metabolism.

If a drug with a substantial first pass effect is formulated as a controlled release product a high percentage of the administered dose will be metabolized, the unchanged drug levels obtained could be continually low, and/or even subtherapeutic, and the bioavailability decreased. If the first pass effect is saturable, it would be found that by increasing the size of the initial and maintenance doses the drug availability might be improved. Nevertheless, in many cases, the

efficacy of such formulations might be questionable. In some other instances, however, a substantial first pass effect may enhance the extent of drug availability. Such is the case of the administration of derivatives of drugs (prodrugs) which are metabolized to active drug molecules. Thus, such degradative processes may be essential for complete bioavailability. As long as the rate limiting step in the administration of prodrugs is its release from the dosage form and not the biotransformation to active drug, their formulation as controlled release product might be advantageous.

D. Advantages and Disadvantages of Controlled Release Preparations

As mentioned previously, some of the most commonly used kinds of controlled release dosage forms are: capsules of coated granules, tablets of coated granules, plastic porous matrices, slow erosion cores with an initial dose and multiple-layer tablets. A survey of how such products have been utilized and the advantages and disadvantages that have been noted with their use follows. The material has been organized according to the dosage form under analysis.

1. Capsules of Coated Granules.

Among the advantages documented for this type of controlled release product are the effective reduction of dosing frequency and better drug dose utilization. For instance, reports on controlled clinical studies showed the equivalency of a 50 mg daily regimen of amitriptyline, administered in controlled release form, Lentizol-Warner, with a 75 mg daily dose of the drug given as conventional tablets, 25 mg t.i.d. (69-72).

The performance of controlled release capsules containing one total daily dose of belladona alkaloids was compared to that of the same

total dose of the drug in three conventional capsules, taken every 8 hours. The results, as measured by the volume of saliva secreted after a standard stimulatory procedure, show that the response following the administration of the controlled release product was equivalent to, and even more uniform than that following the conventional dosing regimen (73). From clinical trials in about 400 patients with different kinds of allergies, Green (74) found that the antihistaminic drug chlorpheniramine hydrochloride given as a controlled release capsule every 12 hrs was as efficacious as the same total daily dose administered 3 or 4 times per day as the conventional product. Better drug dose utilization from controlled release ascorbic acid preparations as compared to the conventional dosage form has also been reported (75).

Disadvantages documented include reduced bioavailability for riboflavin (76) and iron (77) from controlled release products. Iron controlled release preparations have been prepared to reduce g.i. irritation caused by the active ingredient. It has been found (77) that even though the g.i. disturbances have been reduced, the bioavailibility of such preparations was also decreased. Since iron is absorbed with maximum efficiency in the duodenum (78) and the transit time through this intestinal portion might be less than 4 to 6 hours, it appears difficult to manufacture an iron preparation that would effectively produce a sustained absorption. Similarly, the reduced bioavailability of riboflavin from controlled release preparations should have been expected beforehand since it is known that riboflavin is absorbed by an active process localized in the duodenum.

In addition, as an example of incorrect drug formulation, a report

of an evaluation of controlled release preparations of chlorpromazine has appeared (79). As would be expected these studies show therapeutic equivalency between the conventional preparation of chlorpromazine administered three times a day and the same total dose of chlorpromazine as a controlled release dosage form given once a day. However, since the biologic half-life of the drug is 1.29 days (80), there is no rationale for a controlled release preparation of this drug. A similar situation for meprobamate has been pointed out (81).

2. Tablets of Coated Granules.

Advantages documented for this kind of sustained release product include decreased side effects, reduction of dosing frequency and more uniform drug blood levels. Several reports (82 - 84) evaluated the efficacy of a controlled release preparation of aspirin (Measurin-Cheseborough Ponds) in arthritic patients. The blood levels obtained after administration of controlled release tablets every 8 hours were compared to those obtained following the administration of the same daily dose of the drug as conventional tablets every four hours. The controlled release product was found to provide similar blood levels; the peak drug levels were less pronounced, less frequent and of longer duration than those after the administration of the regular tablets. In addition, reduced gastrointestinal disturbances and better control of the night and early morning pains in the arthritic patients were noted. However, Hollister (85) shows data pointing out that when 1.0 g doses of both Measurin and tableted aspirin were compared, drug levels after the controlled release form were very similar to those obtained following the conventional tablet. In addition, Hollister points out that drug bioavailability from Measurin was slightly lower than that from the

conventional form.

As disadvantages, reduced bioavailability for iron (86) and lithium carbonate (87) from this type of controlled release dosage form has been documented. McIntosh et al. (86) report studies conducted with a preparation consisting of ferrous fumarate granules coated with phthalate acetate of cellulose and an initial dose of uncoated granules. These researchers found a lower incidence of g.i. disturbances but also a reduced bioavailability from the tablets of coated granules as compared to the conventional product. It has been explained in a preceding section that since iron is actively absorbed in the duodenal region of the g.i. tract, it is difficult to formulate an iron preparation that would produce a controlled absorption. Caldwell et al. (87) conducted studies to evaluate the efficacy of a lithium carbonate controlled release tablet. A sustained release tablet (C) containing 450 mg of the drug was compared to 300 (A) and 450 (B) mg of the drug as conventional capsules and to a 300 mg dose of the drug in solution (D). All patients received two different preparations, distributed at random, with a 7 day interval between experiments. Blood was sampled over a 24 hr interval and the blood drug level data was utilized to estimate the relative bioavailability of the drug from each of the preparations. The bioavailability obtained from the drug solution represented 100%. The results obtained by Caldwell et al. (87) are summarized as follows:

Formulation	Bioavailability (%)
A	96.0
B	97.4
C	58.1
D (Standard)	100.0

Capsules A and B provided bioavailability of lithium carbonate

equivalent to that from the standard solution. However, preparations A, B and D provided greater bioavailability of the drug than formulation C (at 0.01, 0.05 and 0.05 significance levels, respectively). These results are in agreement with previous findings by other researchers (88, 89).

Additionally, another example of incorrect formulation of a drug in a controlled release dosage form is dealt with in the report of Mellinger et al. (90). These researchers compared drug blood levels following the administration of a controlled release preparation of thioridazine (Thioridazine Spacetabs-Sandoz) to those following the administration of sugar coated tablets and the drug in solution. Identical total doses were compared. As would be expected these studies show equivalency between the three preparations, and provide additional proof of the "prolonged" action of the drug per se. (The reported biologic half-life for thioridazine fluctuates between 30 and 40 hours).

3. Plastic Porous Matrices.

The documented advantages of this type of controlled release dosage form over the conventional products of the drug include the possibility of administration of higher drug doses with no toxic effects, prolongation of the desired drug effect, more uniform drug levels and decreased incidence of side effects. For instance, it has been reported (91, 92) that a controlled release preparation of hyoscyamine allows the administration of considerably higher daily doses (2.7 mg/day) than those in a conventional dosage form (1.6 mg/day) without side effects. Moreover, an effective reduction of the basal gastric secretion was achieved for a period longer than 6 hrs; such effect was attributed to the size of

the dose and its gradual release. Several reports (93-96) have compared the efficacy of oral controlled release plastic matrices of theophylline (Theograd, Abbott)to that of a conventional formulation. Consistently more uniform drug blood levels, within the therapeutic range, are found after administration of the controlled release form than following the conventional form. Similarly, in studies (97) with a controlled release product of quinidine (Duretter), more uniform blood levels have been reported after a Duretter-type quinidine product than after the conventional dosage form. It has also been suggested (97) that the incidence of collateral effects for quinidine might be reduced with the use of such controlled release preparations. No reports on the testing of this possibility have appeared.

Ziehm (98) reports a significant reduction of incidence of side effects, g.i. disturbance, when a controlled release product of iron and ascorbic acid (Ferrograd-500) was used.

There seems to be enough evidence to contraindicate the usage of controlled release KCl after cardiac surgery. Pemberton (99) reports a case of esophageal obstruction developed by a patient ten days after a surgical intervention to replace the mitral valve; the patient was on tablets of "Slow-K." In another case (100) a patient complained of a persistent pain when swallowing, two weeks after correction of Fallot tetralogy surgery; by means of barium as a contrast medium, the presence of a Slow-K tablet in the mid-esophagus was shown. Whitney and Croxon (101) describe 6 cases of patients with left auricular enlargement that presented severe disphagia problems and all of whom developed esophagic constriction. Two of the patients required feeding by a

jejunostomy but died later. One patient died of esophagic ulcer hemorrhage. Five of these patients had been under treatment with the Slow-K product. It has been suggested that the tablets were trapped at the esophageal compression site where they caused an ulceration similar to the well known potassium-induced ileal ulceration. Thus, the contraindication of Slow-K after cardiac surgery seems to be well documented; it is also advisable to avoid the preparation when a cardiac illness, or any other illness requires continuous bed rest.

The report of Hollister et al. (102) provides us with an additional example of incorrect application of controlled release to a drug with inherent "prolonged" action. Sodium pentobarbital in a controlled release form (Gradumet) was compared to the conventional form of the drug. Serum levels were determined and the clinical effects were evaluated after the administration of acute identical doses of each dosage form to a group of 12 patients. From the results shown, it is apparent that the drug serum levels were consistently higher at all times after the administration of the conventional capsules. However, the difference was statistically significant ($p<0.001$) only at the second hour. The authors (102) suggest that drug levels similar to those obtained after the controlled release form could have been obtained after the administration of a simple capsule containing 2/3 of the controlled release dose given. They also point out that even though the Gradumet form provided a delayed absorption, as evidenced by a lower drug level at the 2 hr point as compared to the 4 hr point, it is difficult to visualize this result as having an important clinical advantage.

4. Slow Erosion Nucleus with Initial Dose.

Among the advantages documented for this kind of sustained release dosage form over the conventional products are: more uniform drug levels, reduced dosing frequency, prolongation of the desired effect and decreased incidence of side effects.

Quinn et al. (103) report a study involving identical daily doses of phenmetrazine hydrochloride as two commercial products (three 25 mg capsules) and a 75 mg controlled release preparation (Preludin-Enduret, Boehringer Ingleheim). Drug blood levels were followed for 12 hours. Significant differences between drug levels obtained at 2 hours post-administration were found between the controlled release product and the conventional forms. Six out of 11 subjects complained of sweating and nervousness in the period of 2 to 5 hours after the administration of the conventional products. No complaints of this nature were reported with the use of the prolonged release dosage form.

Herbon and Westwood (104) found that a single tablet of amobarbital in a controlled release form every 12 hours produces a uniform sedation for a 10 to 12 hr interval without the presence of the somnolence periods caused by the same total dose of the drug in conventional tablets administered every 8 hours.

In an evaluation of controlled release tablets (105) containing a total of three doses of mestinon bromide (a neostigmine analog) per unit, a prolonged drug effect for a period of 6 hrs was found. The drug is usually administered every 1 to 3 hrs in conventional products. The authors emphasized the advantage of the controlled release form since no more frequent interruptions of the patients sleep were necessary

for the administration of the drug during the night. Small et al. (106) compared equivalent doses of p-aminosalicylic acid as a solution, buffered conventional tablets and controlled release tablets. A decreased incidence of g.i. disturbances after the controlled release preparation was noted.

5. Multiple-Layer Tablets.

Prolongation of the therapeutic effect and reduced dosing frequency have been reported for this kind of controlled release form when compared to the conventional product. Several investigators (107, 108) have shown no significant therapeutic difference between aspirin 1.3 g controlled release preparations every 12 hrs and aspirin 650 mg conventional tablets every 6 hrs. However, in another study a significant number of patients with rheumatoid arthritis (109) preferred controlled release preparations over the conventional tablet because of better control of morning pain and stiffness. Wiseman (108) reports less fluctuation in salicylate blood levels and a maintenance of these levels for an approximate period of 8 hrs after the administration of controlled release tablets.

Young (110) evaluated the performance of a 10 mg controlled release preparation of triprolidine in a group of 120 patients with a variety of allergic states. Only one tablet was administered per day. General improvement in 91% of the subjects was noted. The main side effect, somnolence, was only noted in 8% of the patients. The antihistaminic effect of the drug was observed for up to 24 hours in most of the cases studied.

E. Pertinent Studies in the Rhesus Monkey

The use of non-human primates in biomedical research has been steadily increasing. Their role in assessing drug toxicity in man has been reviewed (111). Most of the current data indicates that the Old World monkeys usually mimic man from a biochemical standpoint more closely than do other laboratory animals including the New World Monkeys. Data derived almost entirely from studies in rhesus monkeys suggests that from a comparative point of view, metabolic studies in subhuman primates predict the disposition of drugs in man somewhat better than do parallel studies in dogs (112). Based on kidney function, body fluid compartments, water and electrolyte metabolism, the monkey is said to resemble man more than any other experimental animal (113, 114).

Forsyth and coworkers (115) on the basis of previous findings (116), investigated the use of unanesthetized restrained rhesus monkeys in cardiovascular research. Forsyth et al. determined baseline values of several cardiovascular parameters in unanesthetized restrained monkeys, 7 to 10 days after the surgical implantation of chronic catheters in the inferior vena cava and the abdominal aorta of the animals; the results of these studies reinforced the authors' belief that the study of the primate, uncomplicated by anesthesia and recent surgery is of more relevance to man than are the experiments involving more commonly used laboratory animals.

Forsyth et al. (117) have described the normal distribution of cardiac output in the unanesthetized, restrained rhesus monkey. These measurements were made by following the distribution of radioactively labeled microspheres injected into the left ventricle of the heart.

These researchers have demonstrated that the percent of cardiac output transiting specific organs in the monkey are in the same range as those reported in man. For example, the percents of cardiac output in man versus monkey, respectively, for various organs are: liver 28% vs 19%, kidney 23% vs 12%, heart 5% vs 5% and brain 14% vs 7%. In the monkey a much higher total cardiac output per 100 Gm body weight was observed. This high total of cardiac output is reflected in the much higher flow (in blood flow per unit organ weight) seen to most of the regional organs for the monkey as compared to man. This was noticeable (comparing man vs monkey) in flow (ml/min) per 100 Gm of tissue in the liver 58 vs 148, kidney 420 vs 543, heart 84 vs 324 and brain 54 vs 80. It is also noteworthy that these monkeys had an unusually low hematocrit (28.2 to 32.2) compared to the normal value in human subjects (45).

Forsyth and Hoffbrand (118) have shown that there is a redistribution of cardiac output after pentobarbital anesthesia (30 mg/kg dose) in the unanesthetized restrained rhesus monkey. By means of the radioactive microspheres technique, these workers found that anesthesia caused a significant fall in systemic arterial pressure and in total peripheral resistance. Cardiac output, left ventricular and diastolic pressure, and arterial blood pH also fell in each of the anesthetized monkeys, but not enough to be significantly different from the controls. After anesthesia, higher percentages of cardiac output were delivered to the kidneys, skin, lungs (bronchial artery), and bone at the expense of brain, skeletal muscle, adrenals, and chest wall. These latter organs had a significantly decreased blood flow.

Only the lungs had a significantly higher flow, although part of this increase may have been due to arteriovenous shunting of the microspheres in the systemic circulation. The brain was the only organ to have a significantly higher resistance. They also pointed out that in order to avoid these potent and changing alterations during experimental procedures, unanesthetized preparations need to be utilized, wherever possible, in physiologic or pharmacologic studies.

The cardiovascular response to induced emotional stress of the unanesthetized restrained rhesus monkey has previously been studied (119).

Detailed information has been obtained in unanesthetized, restrained male and female rhesus monkeys on the normal lung mechanics, lung ventilation, blood gases and pH (120). It has also been shown that for cynomolgus monkeys, variations in acid-base balance of the blood, in the form of moderate to severe metabolic acidosis, can occur during experimental procedures which may stress the animals (121).

Anatomical features of the rhesus monkey had been reviewed by several authors (122). Scanning electron-microscopic investigation of the luminal surface of the g.i. tract of macaques (123) lead to the conclusion that the epithelial surface of the monkey g.i. tract has very similar features to those found in humans (124).

The gastric secretory response to histamine in the rhesus monkey resembles that observed in man (125). In the basal state the gastric juice obtained through a gastric fistula had a pH above 3.5 and a high pepsin concentration. However, repeated histamine injection led to a rise in free acid and to an increase in pepsin output, in contrast to dogs where no such response with histamine is observed.

Brooks et al. (126) have studied the effect of restraint on fasting gastric contents of spider monkeys during 3 and 24 hour experiments. In the 3 hour restraint experiments, the volume and acid concentration were significantly reduced. The pepsin concentration was increased but not to a statistically significant level. However, in the 24 hour experiments, there was little change noted in the volume and acid concentration when the animals were restrained. Free moving animals showed a greater gastric fluid volume during the period of daytime activity than when they were restrained but showed a marked decrease in the volume and acid concentration during the night.

It has also been shown that the composition of bile in this primate closely resembles the composition of bile in man (127,128). In their studies of the enterohepatic circulation of bile, Dowling et al. (129) were able to exteriorize the normal extrahepatic biliary pathway and by interposing an electronic stream-splitter in the circuit, the normal enterohepatic circulation of bile could be interrupted to an accurately controlled degree. Their results clearly demonstrated that the rhesus monkey could be successfully used, on a chronic basis, to study the enterohepatic circulation of bile and other drugs. Similarly, Mroszczak (130) studied the biliary excretion and enterohepatic circulation of diethylstilbestrol and diethylstilbestrol monoglucuronide in the rhesus monkey.

Meszaros et al. (131) describe a permanent bile fistula preparation utilizing rhesus monkeys as an experimental model that is intended to serve for the determination of intestinal absorption of compounds that are mainly excreted via the bile. Absorption tests were carried out in 9 monkeys using ergotamine tartrate. Recovery of the applied dose

averaged over 96%, while the values for intestinal absorption (% of dose) varied little from animal to animal, the mean ($\pm$ S.D.) being 45 ± 8. These authors also report that the reproducibility of the tests in the same animal over periods of 2-36 weeks was satisfactory, and the results for intestinal absorption revealed a standard deviation of no more than $\pm4\%$.

A variation of the restrained monkey model used by Forsyth and Rosenblum (116) was developed by Nayak and Benet (132, 133) to study the gastrointestinal absorption of drugs and dosage forms in the unanesthetized rhesus monkey. In addition to the chronic vascular catheters that allowed intravenous studies to be carried out and pharmacokinetic parameters of drugs to be determined, gastric and duodenal cannulae were surgically implanted in the monkeys. These cannulae provide a means of instilling a drug solution or a dosage form directly into the stomach or the duodenum. These investigators developed a technique to block the pylorus so that absorption of a drug specifically from the stomach could be studied. Nayak (132) was able to compare the absorption of an ionizable acidic drug, salicylic acid, an ionizable basic drug, amphetamine, and a nonionized drug, antipyrine, from the stomach and the intestine. His results indicate that absorption of all three drugs was faster from the intestine than from the stomach even if the drug was ionized at the absorption site. In contrast to the rate, the extent of absorption of the three drugs from the stomach was almost equal to that from the intestine. Nayak (132) explains that the large absorptive surface area of the intestine as compared to that of the stomach plays an important role in the absorption of drugs. In fact, the surface area differences

can override the effect of the degree of ionization on the rate of absorption.

The stability of the Nayak and Benet preparation (133) was documented by obtaining repeated pharmacokinetic measurements which were unchanged over different lengths of time. In other laboratory (116) similarly prepared monkeys have been shown to have normal blood pressure, heart rate and catecholamine levels for as long as nine months after introduction of vascular catheters. Further illustrations of the value of this model were demonstrated in bioavailability studies of carbamazepine and chlorothiazide (134, 135).

The advantage of the Nayak and Benet model lies in its chronic use and its versatility in the study of different variables such as stomach emptying, food intake, water intake, starvation, and anesthesia, all of which could affect absorption of orally administered drugs. The model allows investigators to isolate specific problems which decrease the bioavailability of a drug from a dosage form, and offers an easily sampled system for measurement of the effect of changes in dosage form design.

The rhesus monkeys prepared as per Nayak and Benet (133) meet most of the requirements set for an animal model suitable for the evaluation of oral controlled release dosage forms: the gastrointestinal system of the monkey is similar anatomically and physiologically to the gastrointestinal system in humans; the preparation allows the investigator to orally administer intact dosage forms without using anesthesia or causing undue stress to the animal; the preparation allows the investigator to sample blood frequently and to run repeated studies

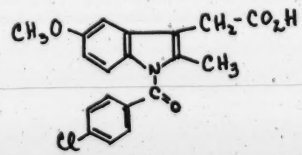
on the same animal and, finally, the preparation is available for long periods of time during the course of each experiment in an unanesthetized state.

Since the small intestine of the rhesus monkey is approximately 80-100 cm long (122) as opposed to approximately 280cm in man (136), a much faster small-intestinal transit time for the monkey would be expected. By adaption of the animal preparation developed by Nayak and Benet (133) to control intestinal transit time, an intact oral dosage form could be kept in contact with the absorbing mucosa for a period of 8-12 hours, thereby constituting an animal model suitable for the evaluation of oral controlled release dosage forms. This work was conducted following such an approach.

F. Indomethacin

1. General Information.

Indomethacin, 1-(p-chlorobenzoyl)-5- methoxy -2- methylindole -3-acetic acid, is a synthetic anti-inflammatory agent with antipyretic and mild analgesic action having the following structure:



Indomethacin was first synthesized by Shen et al. (137) in 1963. It is a weak organic acid with pKa=4.5 and practically insoluble in water. Very little is known about how it exerts its anti-inflammatory effects. Indomethacin can uncouple oxidative phosphorylation (138), inhibit leukocyte migration (139), and possibly alter serum proteins (140). Indomethacin suppresses the vascular permeability-enhancing properties

of bradykinin, which may account for its efficacy during inflammatory conditions with exudative features (141).

Clinically, indomethacin shows the most consistent dramatic benefit in gouty arthritis and osteoarthritis. In many of these patients, analgetic and anti-inflammatory effects are evident within hours of dosing (141). In contrast, dramatic activity is uncommon in rheumatoid arthritic patients where long-term medication is necessary (141). Indomethacin is most commonly administered as 25 mg doses three times daily with meals. The side effects of indomethacin include headaches, dizziness, giddiness, gastric distress, gastrointestinal bleeding, diarrhea, edema, dermatitis and bronchial asthma (142). More serious adverse reactions are the activation of a latent bacterial infection or the masking of signs of an infectious process (141).

2. Pharmacokinetic Data Available in Humans.

a. Absorption.

Indomethacin is rapidly absorbed after oral dosing with peak plasma concentrations by 2 hours. Rectal absorption is also rapid. Appreciable plasma concentrations have been found 15-30 min following the administration of 50 mg capsules or suppositories of the drug (143,144). Complete drug bioavailability from conventional capsules administered both orally and rectally has been shown (143, 144). It has been reported that although the absorption process is initially rapid it remains operative through 8 hours (143).

b. Disposition.

Indomethacin at therapeutic blood levels is 98-99% bound to plasma proteins (145). Indomethacin undergoes extensive O-demethylation and

N-deacylation in man (143). The respective time courses of appearance of the three metabolites (desmethyl-indomethacin, DMI, desbenzoyl-indomethacin, DBI, and desmethyl-desbenzoyl-indomethacin, DMBI) and their glucuronides suggest that the major pathway for the catabolic sequence in man is demethylation (to DMI) mediated by the hepatic microsomal enzyme system, followed by extramicrosomal deacylation (to DMBI), whereas direct deacylation (to DBI) is a competing terminal reaction (143).

The metabolites are devoid of anti-inflammatory activity (146). Indomethacin (I) and its metabolites are excreted in urine and bile both in the free form and as conjugates. An efficient enterohepatic recycling of indomethacin involving secretion into bile as conjugate(s) and reabsorption subsequent to hydrolysis, has been implicated in the intestinal side effects observed in experimental animals and in man (148). The metabolites DMI and DBI are innocuous at dosages 10 times the TD_{50} (Toxic Dose--50) for intestinal lesions resulting from indomethacin (149).

The decay of plasma indomethacin concentrations with respect to time follows a biexponential pattern. The reported half-life values from the β-phase range from 2.6 to 11.2 hrs (144). The apparent volume of distribution (Vd) after a single dose of indomethacin varies between 0.34 L/kg and 1.57 L/kg. The plasma clearances lies between 0.044 and 0.109 L/kg/hr (144). The slower elimination phase can be partly due to enterohepatic recycling. The fecal recovery of indomethacin has been reported to be considerable, i.e., 21 to 61% (143, 150). The enterohepatic recycling might also explain the apparent scatter or

lack of linearity in the log-linear fall-off curves seen in both human volunteers and patients (152).

3. Pharmacokinetic data available in monkeys.

Monkeys extensively metabolize indomethacin to DBI and excrete it in urine. The reported half-life of indomethacin in the monkey is very short, less than 20 minutes (147,151). This rapid fall in plasma concentration might be due to the rapid clearance of indomethacin by the liver into bile since relatively little indomethacin is excreted into urine. Approximately 2-3% of the dose is excreted unchanged in urine in 24 hours (151). The biliary excretion of indomethacin (48% of dose) and its conjugates is extensive (151). After oral administration of indomethacin to monkeys, the apparent half-life is longer (about 90 min), showing that significant absorption is still occurring for at least several hours (147). In 72 hours, less than 10% of the dose is excreted in feces. Of this 10%, DBI represents approximately 60% and indomethacin about 40% (151).

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III. EXPERIMENTAL

A. Animal Set-up

1. Surgical Preparation.

Male rhesus monkeys, 3 to 9.7 kg were utilized. All surgical procedures were performed on fasted animals.

a. Vascular catheterization.

i. Materials:

Sterile polyvinyl tubing (Insultab^R #20, vinyl insulation sleeving, I.D. 0.034" and wall 0.016"; Westglas Co., Burlingame, Ca.), ketamine hydrochloride (Ketalar^R, 100 mg/ml.; Parke-Davis and Co., Detroit, Michigan 48232), sodium pentobarbital (Diabutal^R, 60 mg/ml.; Diamond Laboratories, Des Moines, Iowa 54304), antiseptic cyanide (Antiseptic No.3^R, tablets of mercury cyanide; Eli Lilly and Co., Indianapolis, Indiana 46206), topical antibacterial ointment (Panolog^R, nystatin, neomycin sulfate, thiostrepton and triamcinolone acetonide ointment; E.R. Squibb and Sons, Inc., New York, N.Y. 10022), sodium heparin injection (U.S.P., 1,000 units/ml.; The Upjohn Company, Kalamazoo, Michigan 49001), normal saline solution (0.9% sodium chloride injection, U.S.P.; Travenol Laboratories, Inc., Deerfield, Illinois 60015), surgical instruments as needed.

ii. Methods:

The monkeys were initially immobilized with a 20 mg/kg i.m. dose of ketamine hydrochloride and then anesthetized with a 30 mg/kg i.v. dose of sodium pentobarbital. Polyvinyl catheters, sterilized by soaking them overnight in a 1:500 solution of antiseptic cyanide, were placed under aseptic conditions into the abdominal aorta and inferior vena cava

via the femoral vessels. The surgical procedure is similar to that described by Werdegar, Johnson and Mason (1). A left groin incision was made directly over the femoral pulse and the femoral vein and artery were exposed. Catheters were introduced and passed 12 to 14 cm cephalad into the vein and 6 to 7 cm cephalad into the artery, placing the tip of the catheters in the inferior vena cava and the abdominal aorta distal to the renal arteries. Several ligatures were made around the vessel and the catheter. The distal end of each catheter was curved 180°, sutured onto fascia and tunneled under the skin to the desired exit point near the umbilicus. The incision was closed and a topical antibacterial ointment applied on and around the wound to prevent infection.

b. Gastric cannulation.

i. Materials:

Plastic cannulae for the stomach were made to specification by the Research and Development Laboratory, University of California, San Francisco. The cannula, shown in Fig. 1A, is a modification of that described by Nayak and Benet (2). It consists of an acrylic (Lexam^{R}; Plastic Sales Inc., San Francisco, Ca. 94107) cylindrical tube 4.5 cm long, I.D. 0.8 cm and wall 0.1 cm with a circular flange 0.15 cm thick, diameter 2.0 cm at one end. The cannula can be closed with a screw-in insert, the tip of which remains flush with the flange. Ketamine hydrochloride, sodium pentobarbital and surgical instruments as required.

ii. Methods:

The abdomen was shaved, iodine antiseptic (2% tincture of iodine), was

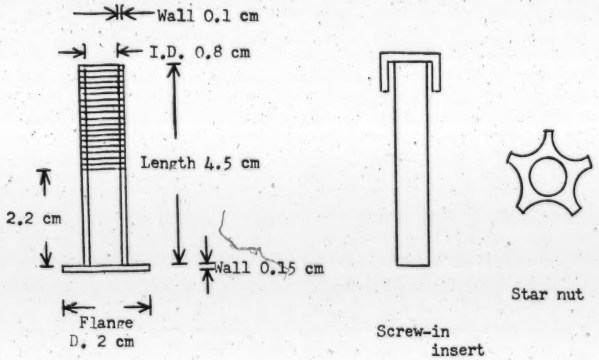

Fig. 1A - Diagram of the Gastric Cannula (G).

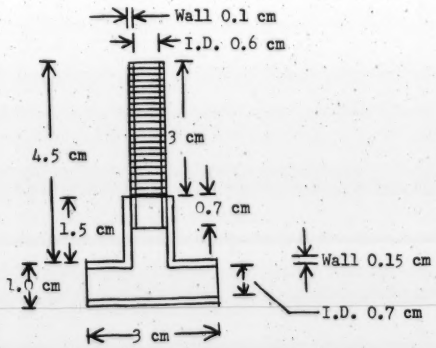

Fig. 1B - Diagram of the Upper Terminal-ileal Cannula (TI).

applied and the field was draped with sterile linen. An upper midline abdominal incision was made and the peritoneal cavity was entered through the linea alba. The stomach was exposed and a gastrotomy incision large enough to introduce the flange of the cannula was made in the mid-region of the stomach near the greater curvature. The cannula was secured in the stomach by means of a purse string suture of 000 silk and exteriorized through a separate stab wound about 4 cm to the left of the mid-line incision. The stomach was sutured to the parietal peritoneum around the stab wound to prevent leakage of gastric fluid into the peritoneal cavity. Star wing nuts were screwed onto the cannula which was stoppered with an insert, the incision was closed and the monkey was allowed to recover from anesthesia.

c. Intestinal Cannulation.

i. Materials:

Intestinal cannulae were made to specification by the Research and Development Laboratory, University of California, San Francisco. The upper-jejunal and lower terminal-ileal cannulae were constructed from acrylic (Lexam^{R}) cylindrical tubing and were 4.5 cm long; the upper-jejunal cannula had an I.D. of 0.6 cm and wall of 0.1 cm The flange at the end of the basic tube was made into an ellipse with a major axis of 2.3 cm and a minor axis of 1.2 cm. The lower terminal-ileal or "blockade" cannula was almost identical to the upper-jejunal cannula, the only differences being the 0.4 cm I.D. and the 0.15 cm wall of the cylindrical tube. The upper terminal-ileal cannula was made of the basic cylindrical tube 3.7 cm long, I.D. 0.6 cm and wall 0.1 cm connected to a T-shaped Silastic^{R} extension with

dimensions shown in Fig. 1B. Each cannula had a screw-in insert with a tip that remained flush with the flange. Ketamine hydrochloride, sodium pentobarbital, penicillin-dihydrostreptomycin (Streptillin^{R}, each ml contains procaine penicillin G 200,000 I.U. and dihydrostreptomycin sulfate equivalent to 0.25 gm streptomycin; Trico Pharmaceuticals, San Carlos, Ca. 94070) and surgical instruments as needed.

ii. Methods:

The animal was immobilized with ketamine hydrochloride and then anesthetized with sodium pentobarbital. One ml of penicillin-dihydrostreptomycin was administered just before the beginning of the surgical operation. A laparotomy was performed on the anesthetized monkey and the small intestines exposed; the upper-jejunum and terminal-ileum were located and marked with a suture; an incision large enough to introduce the flange of the cannula was made in the terminal ileum 5 to 8 cm proximal to the ileocecal junction; the cannula was then implanted and secured by a purse-string suture of 0000 silk. A second cannula was implanted three to five cm proximal to the "blockade" cannula, following the same procedure. A third cannula was implanted in the upper-jejunum. All three cannulas were exteriorized through separate stab wounds, the upper-jejunal to the left and the two ileal to the right of the midline incision about four cm lateral to the upper-jejunal cannula.

d. Ileostomy.

i. Materials:

Methylene blue solution (1% Methylene blue solution in water-alcohol; Aldrich Chemical Company, Inc., Milwaukee, Wisconsin 53233), adhesive

drainable stoma bag with clamp (Karaya seal drainable stoma bag, size 1", medium 16", clamp included; Hollister Inc., Chicago, Illinois 60611), Stomahesive^{R} (peristomal covering, 4"x4" wafer; E.R. Squibb and Sons, Inc., Princeton, N.J. 08540), ketamine hydrochloride, sodium pentobarbital, penicillin-dihydrostreptomycin, surgical instruments and sutures as required.

ii. Methods:

The surgical procedure is similar to that described by Turnbull and Weakley (3). The stoma site was selected by placing a standard ileostomy appliance so that it stayed on a smooth surface, with the upper border just below the umbilicus and the medial border overlapping the midline. The site for the stoma was marked by puncturing the skin with a hypodermic needled dipped in methylene blue solution. A midline incision was used and the small intestines exposed. A mesenteric window was made and the ileum was divided between intestinal clamps. The distal end of ileum was closed with an inner layer of continuous 0000 chromic catgut and an outer layer of interrupted 0000 silk. The closed distal portion of the ileum was placed into the abdominal cavity and attention was then directed to the construction of the ileostomy. A circular 2 cm. diameter disk of skin was excised around the marked ileostomy site and a vertical incision was made through the fat, exposing the rectus muscle sheath. The anterior rectus sheath was incised longitudinally, the muscle fibers were split and the posterior rectus sheath and peritoneum were incised longitudinally. The abdominal wall aperture measured 3.5 cm in diameter. The end of the ileum was grasped with Babcock forceps and drawn through the abdominal wall

aperture. At least 6 cm of ileum, with its mesentery turned cephalad, was delivered through the skin aperture and wrapped with wet gauze. The final external construction of the stoma was deferred for fixation of the intra-abdominal portion of the mesentery. The mesentery was fixed to the peritoneum with interrupted silk sutures. The external construction of the ileal stoma began by passing two 00 cotton ligatures around the mesentery at skin level. The ligatures were tied to secure the mesenteric vessels and the mesentery was divided between the ties. The avascular mesenteric fat was trimmed to reduce the bulk of the stoma in preparation for the final eversion maneuver. The end of the ileum was amputated so that the final everted stoma would be 2 cm in length. Four quadrant 0000 chromic catgut sutures were placed between the end of the ileum and the subcuticular tissue. Slight tension on the quadrant sutures everted the ileum and held the end in juxtaposition to the skin in preparation for the final sutures. The four quadrant sutures were tied and four additional sutures were placed. The stoma was completed.

2. Animal Care and Maintenance.

a. General information and pre-surgical care.

The rhesus monkeys (macaca mulatta) used in this laboratory were acquired through the Primate Import Corporation, New York, which obtains them from India. After arrival the animals are screened and tested for enteritis (Salmonella-Shigella), tuberculosis, Acariasis, Herpes B virus and parasites, in the quarantine period (4, 5). Once an animal has been judged to be clean and adjusted to its new environment, it is moved to the general caging area. The minimum requirement

to be met before any animal can pass into the general colony area is that it has remained in quarantine for no less than 6 weeks and that the animal has been judged to be free of disease and parasites for a minimum of ten days. The animals were fed Purina Monkey Chow^{R} (Purina Monkey Chow^{R} 25; Ralston Purina Co., St. Louis, Mo. 63188), and fresh fruits and water. On an average, monkeys ingest about 20 "bricquettes" daily, (see composition in Table 1, Appendix). The animals were kept in a ventilated, constant temperature room.

b. Post-Operative Care.

Catheterization.

i. Materials:

Penicillin-dihydrostreptomycin, nutritional supplement solution (Ambex^{R}, amino acid solution with electrolytes, vitamin B complex and 5% dextrose, 5 ml/kg i.v.; Elanco Products Company, a division of Eli Lilly and Company, Indianapolis, Ind. 46206), topical antibacterial ointment, normal saline, sodium heparin, Harvard Infusion pump (Model #975; Harvard Apparatus Co., Millis, Massachusetts 02054), primate restraining chair, isolation booth.

ii. Methods:

Post-surgically the monkey was allowed to recover in a supine position after he had been placed in a restraining chair such as described by Forsyth & Rosenblum (6). An external source of heat was placed close to the animal and he was kept on his back until conscious. The monkey was then set upright and the distal ends of both vascular catheters were connected through a luer stub adapter and a three-way stopcock to an infusion pump which was set to constantly infuse 1 ml/hr of a

heparinized saline solution containing 5 units of heparin per ml of saline into each catheter. The restraining chair was placed in an isolation booth. The catheters connected to the infusion pump exit from the booth so that all manipulations could be done without disturbing the animal. Post-surgical infection was prevented by giving one ml. of a penicillin-dihydrostreptomicin injection i.m. every day alternately in each leg for five days (7). His eating and drinking habits were observed and when necessary his diet was supplemented with intravenous nutritional supplement solution. Every other day, a topical antibacterial ointment was applied on and around the wound until perfectly healed. If blood tests warranted it, i.m. Imferon^{R} (Iron-dextran injection, each ml contains 50 mg iron; Lakeside Laboratories, Inc., Milwaukee, Wisconsin 53212), was also administered. The monkey was allowed to recuperate for a period of about 10 days before any experimental protocol or further surgery were attempted.

Gastric and Intestinal Cannulations:

i. Materials:

Same as per catheterization.

ii. Methods:

Same as per catheterization and in addition, daily application of topical antibacterial ointment around the cannula(e) to prevent infection. In general, the monkey was not used experimentally for at least 10 days after surgery.

Ileostomy:

i. Materials:

Same as for catheterization. In addition: Hartmann's solution

(lactated Ringer's injection, U.S.P.; Travenol Laboratories, Inc., Deerfield, Illinois 60015), adhesive drainable stoma bag with Karaya seal and clamp, ostomy belt (Hollister ostomy belt, infant size; Hollister, Inc., Chicago, Illinois 60611), peristomal covering, (Stomahesive^{R}).

ii. Methods:

Same as per catheterization. In addition, immediately after the stoma was completed, its surroundings were thoroughly cleansed, dried and a peristomal covering (Stomahesive^{R}) applied as follows: a hole 1 cm smaller than the stoma was cut in the Stomahesive^{R} wafer, 6 equally spaced 0.5 cm radial slits were cut. The radial slits allow the wafer to form a 0.5 cm up-turned lip which fits snugly around the base of the stoma without causing pressure. The wafer was cut in a circular shape and size so as to cover about 2 cm of surface around the stoma. The adhesive back of the stoma bag was cut to the same size and shape as the Stomahesive wafer, applied to the "shiny" surface of the wafer and smoothed out. The sticky surface of the wafer was positioned over the stoma with application of gentle pressure for 30 seconds. An infant size ostomy belt was attached to the stoma bag and tied around the monkey's waist. The bag was then in place. Hartmann and normal saline solutions were intravenously administered to the monkey as needed in order to maintain his serum electrolyte levels in the normal range. Additionally an intravenous nutritional supplement solution was given as needed (7).

c. Maintenance.

Vascular Catheters:

i. Materials:

Normal saline solution, sodium heparin solution.

ii. Methods:

As pointed out in the post-operative care section, the catheters are constantly infused with a slightly heparinized saline solution at a rate of 1 ml/hr. In addition, every day the catheters are manually flushed with about 5 ml. of heparinized saline to insure that no clots develop. Using this procedure the catheters can be kept functional for months. When the catheters become clogged, they can be removed from the left iliac vessels and new catheters can be introduced on the right side (7, 8).

Gastric and Intestinal Cannulae:

i. Materials:

Topical antibacterial ointment, isotonic saline solution.

ii. Methods:

The gastric cannula is practically self-maintaining; only external cleaning and occasional application of topical antibacterial ointment was required. The intestinal cannulae tend to become infected more easily, therefore rinsing of their surroundings with isotonic saline solution and application of the topical antibacterial ointment was performed more frequently.

Ileostomy:

i. Materials:

Same as those mentioned in the ileostomy post-operative care section. In addition, Hollister^{R} Medical Adhesive (Dow Corning medical adhesive B, Polysiloxane pressure-sensitive adhesive polymer dissolved

in fluorocarbon-propellant; Hollister Inc., Chicago, Illinois 60611), Hollister^{R} Remover (Dow Corning Remover, 97% fluorocarbon solvent with 3% gas propellant; Hollister Inc., Chicago, Illinois 60611), Hollister^{R} Skin Gel (a peristomal protective film; Hollister Inc., Chicago, Illinois 60611).

ii. Methods:

The bag should be emptied and cleaned at least twice daily; however, the likelihood of its becoming loose should be kept in mind and therefore, careful examination would determine if a new bag should be applied. If that is the case, the bag is removed, the skin around the stoma is thoroughly rinsed with isotonic saline solution, dried and then a new bag is applied following the procedure mentioned in the post-operative care section. With time the skin around the stoma might become brittle and then the application of a peristomal protective film (Hollister^{R} Skin Gel for Ostomates) is recommended. A non-injurious medical adhesive aerosol (Hollister Medical Adhesive) is ideal for adhering ostomy appliance. It is applied following the package insert directions. When changing the ostomy bag, the Hollister^{R} Remover is most helpful.

In addition, care must be exercised in maintaining electrolyte balance in the monkey. Input of electrolyte solution (Hartmann's and normal saline solutions) is titrated versus output of electrolytes in urine and intestinal fluids so as to maintain serum electrolyte levels in the normal range. It is also recommended that the eating habits and body weight of the animal be followed carefully and when necessary, intravenous nutritional supplement should be administered.

3. Model I: Multicannular Monkey.

a. Preparation of the animal.

Surgical procedures for the catheterization (Section III.A.1.a), the gastric cannulation (Section III.A.1.b), and the three intestinal cannulae implantation (Section III.A.1.c) were followed.

b. Description.

Model I consists then of a monkey with chronic arterial and venous catheters, one gastric cannula (G), one "blockade" cannula (B) at the ileocecal level, one additional cannula in the upper-jejunum (UJ) and another in the terminal ileum, (TI). By insertion of a foley catheter 8Fr. with a 3cc balloon into cannula B and inflating this balloon inside the lumen of the small intestine, the passage of intestinal contents to the large bowel could be prevented. The external ends of cannulae UJ and TI could be connected to each other by means of latex tubing. A schematic representation of the relative location of the cannulae in the gastrointestinal tract of the monkey is shown in Fig. 2. Figure 3 is a photograph of the fully prepared multicannular monkey.

4. Model II: Ileostomized Monkey.

a. Preparation of the animal.

Surgical procedures for the catheterization (Section III.A.1.a), the gastric cannulation (Section III.A.1.b), the UJ intestinal cannula implantation (Section III.A.1.c), and the ileostomy (Section III.A.1.d) were followed.

b. Description.

Model II consists of a monkey with arterial and venous catheters, one

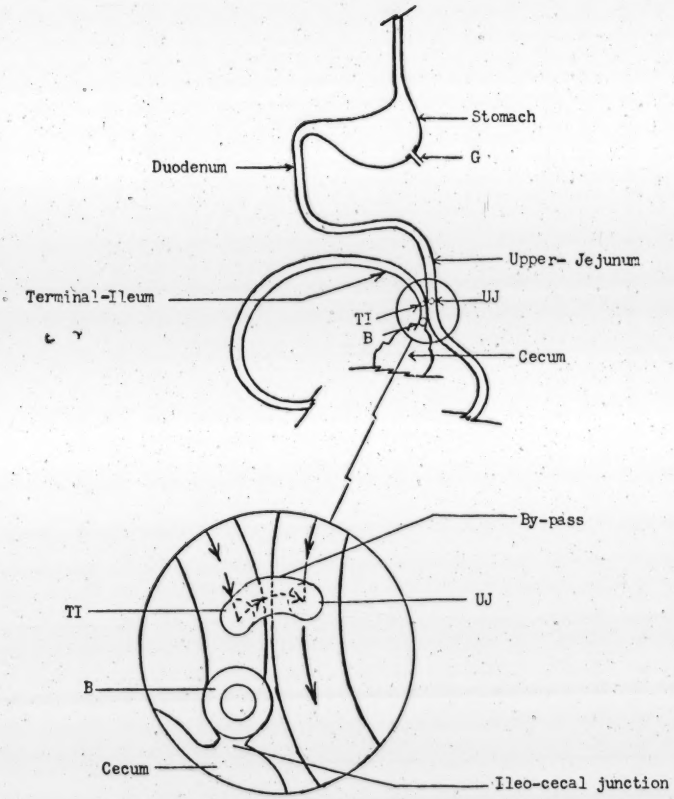

Fig. 2 - Schematic Representation of the Relative Location of the Cannulae in the Gastrointestinal Tract of Monkeys Prepared as per Model I.

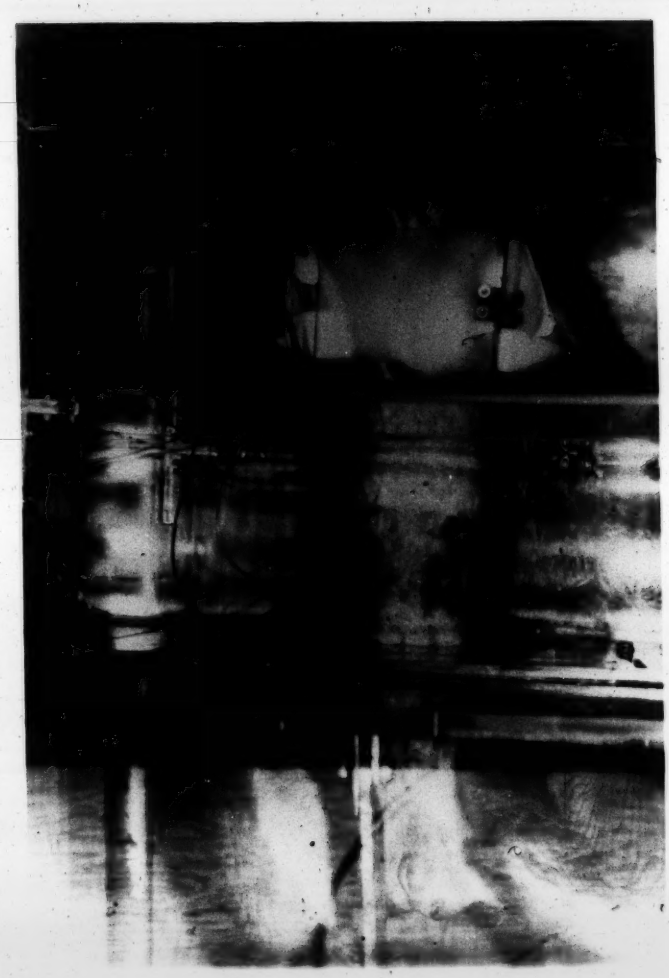

Fig. 3 - Photograph of the Abdominal Region of a Fully Prepared Multicannular Monkey (Model I).

gastric cannula (G), one intestinal cannula (UJ) and an ileostomy (IL). Figure 4 is a schematic representation of the location of G, UJ and IL in the gastrointestinal tract of the monkey.

A photograph of the ileostomized monkey is shown in Fig. 5; Figure 6A shows the abdominal region of a Model II preparation in detail. The stoma and stoma appliance in place are shown in Figures 6B and 6C, respectively.

B. Quantification of Indomethacin

1. Assay Procedure.

A modification of the method of Hucker et al. (9), developed by Drs. Wynosky, Porter and Grabowski of Merck, Sharp and Dohme Research Laboratories (unpublished), was used for the quantification of indomethacin in plasma, urine and ileal fluid specimens.

a. Materials:

Pure indomethacin (Lot #F146032), (I), donated by Merck, Sharp and Dohme Research Laboratories, West Point, Pa.; sodium phosphate buffer, pH=8.0; 1/15 M monopotassium phosphate (KH_2PO_4, crystals, reagent grade); 1/15 M disodium phosphate (Na_2HPO_4, anhydrous, reagent grade); indomethacin daily standards: 0.1, 1 and 10 mcg/ml; heptane (analytical reagant grade) with 3% isoamyl alcohol (reagent grade); 0.01 M cupric chloride ($CuCl_2.2H_2O$, crystals, reagent grade); 0.2M sodium carbonate (anhydrous, reagent grade); 0.1 mM cupric chloride in 0.2M sodium carbonate; 0.5M citrate buffer, pH=5.0; 5N NaOH (U.S.P. pellets); Fluorescence Spectrophotometer 203, Perkin Elmer; Cahn Gram Electrobalance; Research pH Meter, Model 1019, Beckman Instruments; Mettler H10T balance (Mettler Instrument Corp., Princeton, New Jersey 08540);

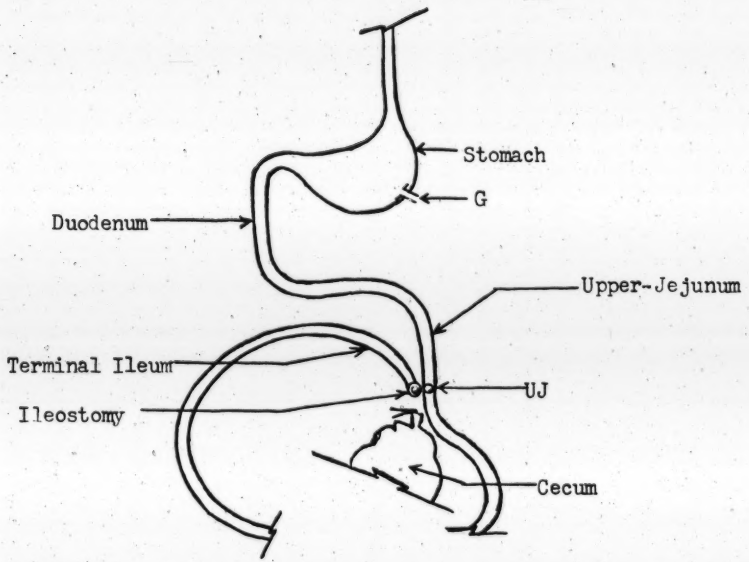

Fig. 4 - Schematic Representation of the Location of the Cannulae and the Ileostomy in Model II Preparations.

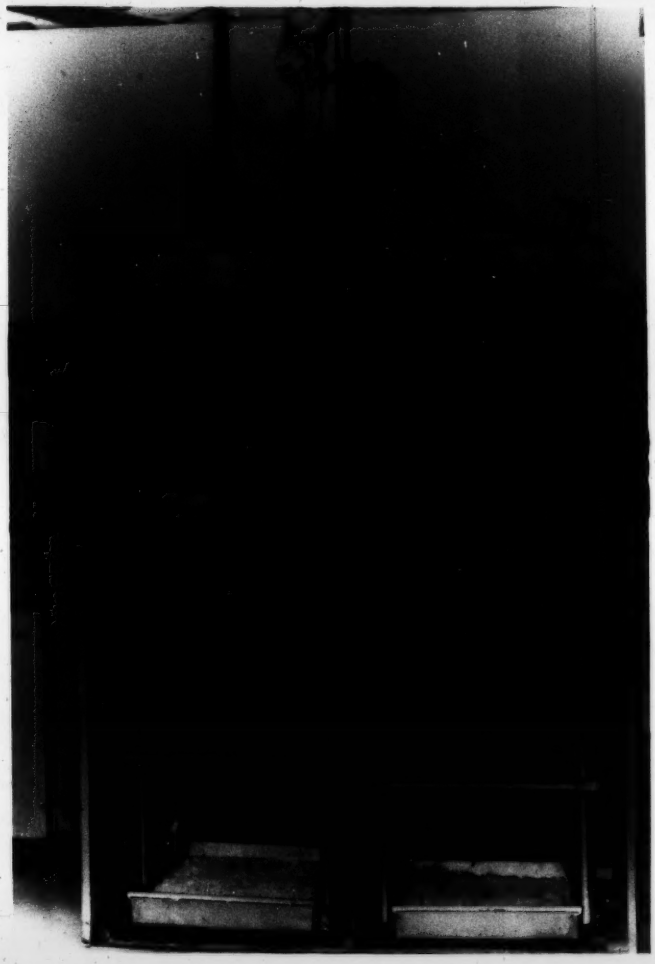

Fig. 5 - Photograph Showing One Model II Preparation (left) in the Restraining Chair and the Isolation Booth Set-up.

Fig. 6A - Detail of the Abdominal Region of a Model II Preparation Showing Cannulae G, UJ and the Ileostomy Covered with an Ileostomy Appliance.

Fig. 6B - Photograph Showing the Uncovered Ileostomy in a Rhesus Monkey.

Fig. 6C - Photograph Showing a Model II Preparation with the Ileostomy Appliance in Place.

International Centrifuge, Model HN-S (International Equipment Co.); "Tilt-type" Mixer (Linson Instruments, Stockholm, Sweden).

b. Methods:

i. Preparation of solutions:

1/15 M Monopotassium phosphate: 0.9 g KH_2PO_4 per 100 ml.

1/15 M disodium phosphate: 9.5g Na_2HPO_4 per liter.

Sodium phosphate buffer, pH=8.0: to 945 ml of 1/15 M disodium phosphate, add 1/15 monopotassium phosphate until a solution pH of 8.0 is reached (about 55 ml needed).

0.5M Citrate buffer, pH=5.0: Place 21.5g citric acid monohydrate ($HOC(COOH)(CH_2COOH)_2$ reagent), and 47.0 g sodium citrate ($Na_2C_6H_5O_7 \cdot 2H_2O$, crystals, reagent), in a liter volumetric flask and fill to the mark with distilled water. Add 5N NaOH dropwise until pH reads 5.0. Keep refrigerated.

0.1mM Cupric chloride in 0.2M sodium carbonate: to 100 ml of 0.2M sodium carbonate, add 1 ml of 0.01 M $CuCl_2$. Prepare fresh daily.

Standards in sodium phosphate buffer, pH=8.0: indomethacin stock solution 100 mcg/ml: place 10 mg of indomethacin in a 100 ml volumetric flask, add about 40 ml of the sodium phosphate buffer, pH=8.0 and place over a steam bath for five minutes. Let the solution cool to room temperature and dilute to the 100 ml mark with sodium phosphate buffer, pH=8.0. Prepare 0.1, 1 & 10 mcg/ml indomethacin standards by dilution daily.

Heptane with 3% isoamyl alcohol: heptane is washed successively with 1N NaOH, 1N HCl and water before using, then isoamyl alcohol is added to make a 3% v/v solution.

ii. Assay technique:

One milliliter of the sample is pipetted into a 50ml glass-stoppered centrifuge tube containing 25 ml of heptane with 3% isoamyl alcohol and 2.0 ml of 0.5 M citrate buffer, pH=5.0. For the standard curve, 1 ml of the appropriate standard solution is also added. The tube is shaken for 15 minutes and centrifuged for 10 minutes at 1,600 r.p.m., then 20 ml of the organic phase (top layer) is transferred to a new tube containing 20 ml of 0.5M citrate buffer, pH=5.0. The tube is shaken for 10 minutes and centrifuged for 10 minutes at 1,600 r.p.m. The aqueous phase (bottom layer) is removed and then 20 ml of 0.5 M citrate buffer, pH=5.0 added to the remaining organic phase. The tube is shaken and centrifuged again for 10 minutes. Fifteen ml of the organic phase (top layer) is then transferred to a new tube containing 4 ml of a solution of 0.1 mM $CuCl_2$ and 0.2 M Na_2CO_3. The tube is shaken and centrifuged for 10 minutes; then the organic phase (top layer) is aspirated and discarded. The alkaline phase is transferred to a test tube and read on the fluorometer, at an excitation wavelength of 300 nm and an emission wave-length of 370 nm. Meter wavelength readings are uncorrected.

2. Assay Reproducibility.

a. Materials:

Same as per assay procedure.

b. Methods:

A 100 mcg/ml indomethacin stock solution was prepared and from this 10,1 and 0.1 mcg/ml solutions were prepared. Five samples of each of the indomethacin standards, including the 100 mcg/ml solution, were

then prepared. One ml of blank plasma was added to each test tube and the assay technique as per the preceding section was followed.

i. Stock solutions of concentrations 1, 10 and 100 mcg/ml indomethacin were tested for stability by placing them in the refrigerator for 1 and 3 weeks.

ii. Five sets of frozen plasma samples at 1, 10 and 100 mcg/ml were also tested for stability over a three week period.

3. Assay Specificity.

a. Materials:

Same as per assay procedure. In addition: O-desmethyl-indomethacin (DMI), L-594,947-00R06 and N-deschlorobenzoyl-indomethacin (DBI) L-560,081-00Z08, donated by the Merck, Sharp and Dohme Research Laboratories, West Point, Pa.

b. Methods:

Preparation of solutions:

Same as per (III.B.1.b.i); stock solution of I, 100 mcg/ml. Stock solution of DBI, 100 mcg/ml and stock solution of DMI, 100 mcg/ml, in sodium phosphate buffer, pH=8.0 were prepared; from these, the following dilutions were prepared: for I: 10, 1 and 0.1 mcg/ml; for DBI and DMI: 10 and 1 mcg/ml. The samples were prepared as follows:

Sample No.	Contents
1:	1 ml. of Phosphate buffer pH=8.0
2:	100 mcg/ml I
3:	10 mcg/ml I
4:	1 mcg/ml I
5:	0.1 mcg/ml I

Sample No.	Contents
6:	100 mcg/ml I + 100 mcg/ml DBI + 100 mcg/ml DMI
7:	100 mcg/ml I + 10 mcg/ml DBI + 10 mcg/ml DMI
8:	100 mcg/ml I + 1 mcg/ml DBI + 1 mcg/ml DMI
9:	10 mcg/ml I + 100 mcg/ml DBI + 100 mcg/ml DMI
10:	10 mcg/ml I + 10 mcg/ml DBI + 10 mcg/ml DMI
11:	10 mcg/ml I + 1 mcg/ml DBI + 1 mcg/ml DMI
12:	1.0 mcg/ml I + 100 mcg/ml DBI + 100 mcg/ml DMI
13:	1.0 mcg/ml I + 10 mcg/ml DBI + 10 mcg/ml DMI
14:	1.0 mcg/ml I + 1 mcg/ml DBI + 1 mcg/ml DMI
15:	0.1 mcg/ml I + 100 mcg/ml DBI + 100 mcg/ml DMI
16:	0.1 mcg/ml I + 10 mcg/ml DBI + 10 mcg/ml DMI
17:	0.1 mcg/ml I + 1 mcg/ml DBI + 1 mcg/ml DMI

One ml of plasma was added to each sample and the assay technique as per (III.B.1.b.ii) was followed.

C. Animal Studies

1. Radiological.

a. Gastrointestinal transit time.

i. Viscous suspension:

Pre-surgery:

Materials:

$BaSO_4$ suspension (Rediflow^R, Barium Sulfate 18% w/w; Flow Pharmaceuticals, Inc., Palo Alto, Ca. 94303), nasogastric tube (Bardex^R 12 Fr., red rubber; C.R. Bard Inc., Murray Hill, New Jersey).

Modified primate restraining chair: The chair as described by Nayak and Benet, and Forsyth and Rosenblum (2, 6) was modified so as to

eliminate the interference of metallic bars when X-ray photos of the gastrointestinal tract of the monkey were to be taken. Slots of 23 cm length and 1.4 cm width were cut through the two lower horizontal acrylic boards of the primate restraining chair. These slots were located 14.5 cm from the front and 5.5 cm from both sides of the acrylic board as the monkey faces the investigator. X-ray film (Kodak RP-X-OMAT, medical X-ray film used with Radelin TF-2 high speed screen; Eastman Kodak Co., Rochester, New York).

Methods:

The animal was fasted overnight having free access to water. One dose of 0.5 to 1.0 ml of ketamine hydrochloride was administered intramuscularly to immobilize the animal for transfer from the cage to the modified primate restraining chair. No additional anesthetic was administered for the placement of the nasogastric tube. An initial, "scout", X-ray plate (factors 2.5 MaS, 70 KV) was taken. A 50 to 60 ml volume of the barium sulfate suspension was given and X-ray plates were taken at intervals of 15 minutes during the first hour and 30 minutes thereafter until most of the administered radiopaque medium passed to the cecum. Food was not allowed during the studies.

Post-surgery:

Materials:

$BaSO_4$ suspension, modified restraining chair, foley catheter (Bardex^R foley catheter, size 14 Fr. with 5cc balloon; C.R. Bard Inc., Murray Hill, New Jersey).

Methods:

The animal was fasted overnight, water was allowed ad libitum. Fifty to sixty ml of a $BaSO_4$ suspension was administered via the gastric

cannula by means of a foley catheter with a 5cc balloon. X-ray plates were taken following the same protocol as prior to the surgical preparation of the animal. The animal was not fed during the study.

ii. Small solid particles:

Pre-surgery:

Materials:

Radiopaque "beads" (rings, 2mm O.D. x 0.5mm width, cut from a "Lehman" catheter #7 Fr., made of woven Dacron with a special radiopaque coating; USCI, a Division of C.R. Bard, Inc., Glens Falls, N.Y. 12801), normal saline, modified restraining chair, nasogastric tube 12 Fr., ketamine hydrochloride.

Methods:

The animal was fasted overnight, water allowed ad libitum. No food was allowed during the study. Ketamine hydrochloride, 0.5 to 1.0 ml, was administered i.m. to immobilize the animal and facilitate transfer of the monkey from the cage to the modified restraining chair. Fifty to one hundred radiopaque "beads" suspended in 50 to 60 ml of normal saline were administered to the monkey via a nasogastric tube. X-ray plates were taken as before. The transit time was recorded.

Post surgery:

Materials:

Radiopaque "beads", normal saline, modified restraining chair, foley catheter.

Methods:

The animal, Model I or Model II, was fasted overnight; water was allowed ad libitum. Food was withheld during the study. Fifty to one hundred

radiopaque "beads" suspended in 50 to 60 ml of normal saline were administered via the gastric cannula by means of a 14 Fr. foley catheter with a 5cc balloon. X-ray plates were taken as before. The transit time was noted.

iii. Radiopaque non-disintegrating cores:

Materials:

Hard gelatin capsules (sizes #1 and #3) filled with $BaSO_4$ and stearyl alcohol in a non-disintegrating core (samples N1 and N2, respectively), donated by Merck, Sharp and Dohme Research Laboratories, West Point, Pa.; modified restraining chair:

Methods:

The monkey prepared with vascular catheters and gastric cannula was fasted overnight, water allowed ad libitum. No food was allowed during the study. The sample, N1 or N2, was administered to the animal via the gastric cannula, (G). X-ray plates were taken as mentioned previously and the transit time noted.

b. Effectiveness of blockade at ileocecal level.

Materials:

$BaSO_4$ suspension, one 14 Fr. foley catheter and one 8 Fr. foley catheter (Bardex^R foley catheter, with coating containing Teflon, size 8 Fr. with 3cc balloon; C.R. Bard, Inc., Murray Hill, New Jersey).

Methods:

In Model I, the passage of contents in the small intestine to the large bowel was prevented by insertion of a foley catheter 8 Fr. into cannula B and inflating the 3cc balloon inside the lumen of the small intestine. Ten to twenty milliliters of $BaSO_4$ suspension were

administered through cannula TI by means of another foley catheter. X-ray plates were taken at intervals of 15 minutes during the first hour and 30 minutes thereafter for 4 hours.

c. Effectiveness of ileo-jejunal connection.

Materials:

$BaSO_4$ suspension; latex tubing, length 14.5 cm, 0.7 cm I.D. and 0.15 cm wall; 2 foley catheters (14 Fr. and 8 Fr.).

Methods:

The animal, Model I, was fasted overnight, water allowed ad libitum. Food was withheld during the study. Passage to large bowel was prevented as described above. Cannulae UJ and TI were connected to each other by means of latex tubing. Fifty to sixty ml of $BaSO_4$ suspension were administered through the gastric (G) cannula. X-ray plates were taken as before, for 6 hours.

2. Indomethacin.

a. Intravenous solution.

i. Materials:

Indomethacin in sodium phosphate buffer, pH=8.0; Millipore Disposable Filter Unit (Millex^R, Sterile 0.22μ, pressure 5 bars; Millipore Corporation, Bedford, Massachusetts); heparinized test-tubes (Vacutainer^R, green stopper, heparinized-evacuated glass tube, sodium heparin 143 U.S.P. units/tube; Becton-Dickinson, Div. of Becton, Dickinson and Company, Rutherford, New Jersey 07070); timer (Lab-Chron Timer^R, minutes and hundredths; Lab-Line Instruments, Inc., Melrose Park, Illinois, 60160); infusion pump; urine-collection pan (made of stainless steel to specifications by the School of Pharmacy Shop, University of California, San Francisco).

ii. Methods:

Indomethacin solutions in sodium phosphate buffer pH=8, were administered to the monkey via the venous catheter at doses of 5 mg/kg of body weight. The total dose was dissolved in the volume of buffer required to make solutions of 2 to 4 mg/ml of indomethacin. The solution of the drug was transferred to a syringe and a disposable filter unit was attached to the syringe tip. The infusion pump was set at such a rate as to allow the administration of the total volume of solution in 2 minutes. The drug solution was administered and the timer started. Two milliliters blood samples were collected via the arterial catheter at various time intervals, usually at 0, 0.083, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3, 4, 6, 8 and 24 hours after administration and transferred to a heparinized test tube. The sample was centrifuged at 2,500 r.p.m., a 1 ml aliquot of plasma was taken and transferred to a clean test tube. Pooled urine samples from 0-8 and 8-24 hours were collected in a feces-separating pan. The volume of each sample was measured and a 1 ml aliquot of each sample was then taken and transferred to a clean test tube. The plasma and urine aliquots were stored immediately after collection in a -15°C freezer, until assayed for indomethacin.

b. Oral Solution.

i. Materials:

Ninety or one hundred milligrams of indomethacin solubilized in 25 or 50 ml of sodium phosphate buffer, pH=8.0; one foley catheter 14 Fr. (Bardex^{R} foley catheter size 14 Fr. with 5cc balloon; C.R. Bard Inc., Murray Hill, New Jersey); as required for the collection of blood and urine samples.

ii. Methods:

The animal was fasted overnight and water allowed ad libitum at all times; food was withheld throughout the study. The drug in solution was administered to the animal via the gastric cannula and the timer started. Two milliliter blood samples were taken at 0, 0.83, 0.167, 0.25, 0.33, 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 6, 8 and 24 hours after administration of the drug. Pooled urine samples from 0-8 and 8-24 hours were collected recording the volume of each sample. One milliliter aliquots of plasma and urine were frozen for storage until assayed for indomethacin.

c. Oral Conventional Capsules (In-line indomethacin capsules).

i. Materials:

In-line indomethacin Capsules (25 mg each; Merck, Sharp and Dohme Research Laboratories, West Point, Pa. 19486); "Recycling" 20 Fr. foley catheter (Bardex^{R} foley catheter, size 20 Fr. with 5cc balloon; C.R. Bard Inc., Murray Hill, New Jersey); the remainder as per oral solution.

ii. Methods:

Three or four capsules of indomethacin were administered to the fasted animal via the gastric cannula. Blood and urine samples were collected following the experimental protocol described previously for the oral solution; in addition, the contents of the small intestine at the terminal ileum were collected and recirculated back into the mid-jejunum.

Recycling Procedure:

Model I: A foley catheter 8 Fr. was inserted in cannula B and the balloon was inflated inside the lumen of the small intestine blocking

the passage to the large bowel. A piece of latex tubing was attached to the external end of the TI cannula and an additional foley catheter 20 Fr. was inserted and then inflated inside the UJ cannula. After administration of the drug, the intestinal fluids coming out from the latex tubing at TI were collected and re-administered to the monkey through the UJ cannula by means of the foley catheter.

Model II: A 20 Fr. foley catheter was inserted and then inflated inside the UJ cannula. The intestinal fluids were continually collected in the ileostomy bag. When a volume of 20 to 25 ml of fluid had been collected in the bag, the volume was adjusted to 25 ml. with saline solution. A 1 ml aliquot was taken and then the remaining 24 ml was readministered via the UJ cannula. The time of collection was noted. The intestinal output collected in the stoma bag at the 8th hour was sampled but notre-administered to the monkey. Aliquots were frozen for storage until assayed for indomethacin.

d. Oral Controlled Release Capsules.

i. Materials:

Indomethacin (Controlled released capsules, dose equivalent to 75 mg of indomethacin, samples P1, P2, P3 (plastic matrices), G1 and G2 (coated granules); Merck, Sharp and Dohme Research Laboratories, West Point, Pa. 19486).

ii: Methods:

Seventy five milligrams of indomethacin in a controlled release formulation was administered to the fasted monkey. The experimental protocol followed was as per oral conventional capsules.

D. References

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IV. RESULTS

A. Animal Set-up

Care and Maintenance.

1. Model I.

Four monkeys were prepared as per Model I; no body weight changes were found in these monkeys during the course of the experiments. Usually within ten days after the implantation of the gastric and intestinal cannulae the monkeys would recover completely from the surgical trauma. In one case however, the monkey never returned to his pre-surgical feeding and drinking habits and then, 8 days after the surgery had been performed, intestinal contents began to seep out from around the two ileal cannulae. The animal was sacrificed and a necropsy was performed; localized infection at the ileal cannula site was observed.

The vascular catheters and the gastric cannula presented no problem whatsoever, being functional at all times in Model I preparations. The intestinal cannulae however, were subject to an extrusion reaction by the monkey. The cannulae were retained in the gastrointestinal tract of the animal for an average of 36 (S.D.=27) days. In all cases the TI cannula was the first to be extruded, followed by the B and then the UJ cannulae. Cannula extrusion was usually preceded by a 2 to 4 day period in which a slight leakage of intestinal fluids from the fistula around the cannula would be observed. The extrusion reaction would culminate with the cannula slipping out.

In one instance, where the extrusion of the cannulae may have been caused by a blockade of the small bowel by intestinal contents, radiological studies were undertaken. A $BaSO_4$ suspension was given

through cannula G and its transit followed by means of X-ray plates. No obstruction of the small intestine was found. Table I shows individual body weights and extrusion reaction times for the four Model I preparations.

2. Model II.

Four monkeys were prepared as per Model II. The post-operative period in Model II preparations seemed to be of critical importance; two out of four monkeys never adjusted to the changes brought by the surgery. In most of the prepared animals, death was attributed to electrolyte imbalance and/or malnutrition. In one instance a body weight decrease of 26.7% was recorded from the date of surgery to the death of the monkey, ten days later. Table II shows individual body weight and post-surgery survival time of the four Model II preparations. The numbers in parenthesis in column 3 indicate the number of days after surgery at which the upper jejunal cannula slipped out from the body of the monkey. Table III shows serum electrolyte levels for monkeys E & J, before and after the ileostomy. Tables IV and V show the electrolyte levels found in urine and ileal contents respectively in Monkey J after the ileostomy.

B. Quantification of Indomethacin

1. Assay Reproducibility

Determination of indomethacin in each of the samples prepared from the standard solutions (100, 10, 1 mcg/ml: 5 samples each; 0.1 mcg/ml: 3 samples) gave the following results as shown in Table VI. The linear standard curves prepared always had correlation coefficients higher than 0.992. Standard curves with concentrations ranging from 0.05 to

TABLE I. Extrusion Reaction Time for Model I Preparations.

MONKEY	BODY WEIGHT (Kg)	EXTRUSION REACTION TIME (days)
B	6.4	28.
C	5.4	38.
D	5.4	72.
F	9.7	8.*
MEAN	6.7	36.
S.D.	2.0	27.

* Never Recovered from Surgery

TABLE II. Post-Surgery Survival Times for Model II Preparations.

MONKEY	BODY WEIGHT (Kg)	POST-SURGERY SURVIVAL TIME (days)
A	8.6	19*
G	6.2	44 (44)**
E	8.5	24 (19)
J	4.7	10*
MEAN	7.0	24
S.D.	1.9	14

* Never Recovered from Surgery

** Numbers indicate days after surgery at which the upper jejunal cannula was extruded from the body of the animal.

TABLE III. Serum Electrolyte Levels (mEq/L) for Monkeys E and J.

Electrolytes	Pre-Surgery *			Post-Surgery							
				Days After Surgery							
				E		J					
	Reported Values In Literature (1 , 2).	E	J	14	24	4	5	6	7	8	9
Na^+	133-148	148	132	123	121	144	141	144	138	136	138
K^+	3.5-6	4.2	3.8	5	4.8	3.8	3.5	3.3	2.9	3.7	3.6
Cl^-	102-117	109	103	99	100	106	100	101	103	105	109
HCO_3^-	-	-	25	15	11	22	26	25	23	16	15
Anion Gap	-	-	17	14	14	19	18	21	15	19	17

*Surgery: as per Model II.

TABLE IV. Urine Electrolytes (mEq/L) in Monkey J.

	Pre-Ileostomy	Post-Ileostomy				
		Days After Ileostomy				Reported Changes In Man, (3).
		6	7	8	9	
Na +	63.	69	80	33	28	↓
K +	72.	56	29	34	27	↓
Cl -	63.	84	70	59	47	↓
Specific Gravity	1.025	1.024	1.011	-	-	↓

TABLE V. Ileal Fluid Electrolytes after the Ileostomy (mEq/L), in Monkey J.

	Days Post-Ileostomy			Values Reported in Literature for Man, (3, 4).
	6	8	9	
Na +	140	117	113	120-130
K +	20	30	43	15-40
Cl -	53	46	34	110

TABLE VI. Indomethacin Assay Reproducibility.

Concentration (mcg/ml)	Mean	Standard Deviation	Coefficient of Variation
100.0	100.7	1.30	1.29%
10.0	9.95	0.37	3.72%
1.0	0.98	0.03	3.47%
0.1	0.106	0.013	12.38%

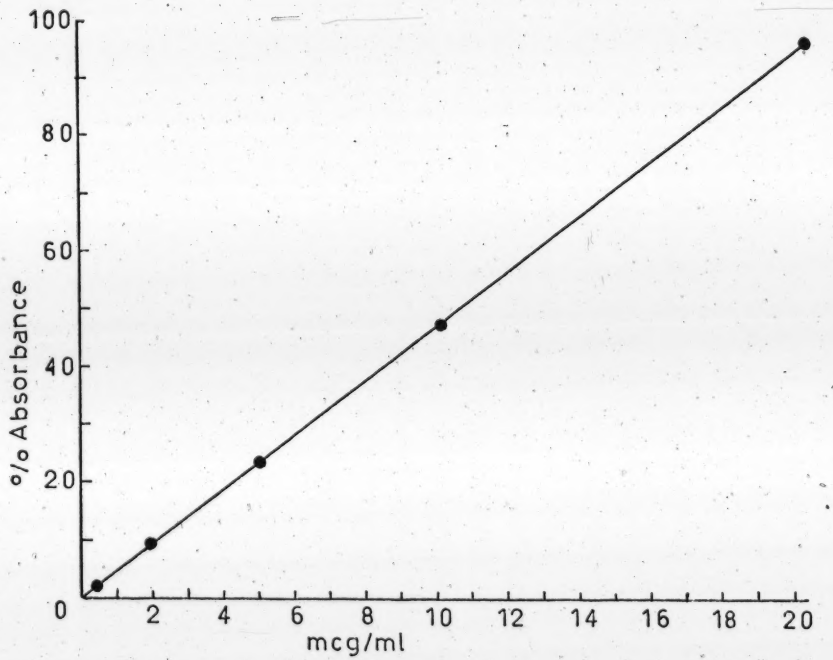


Fig. 7 - Example of Standard Curve of Indomethacin for Plasma Concentration Ranges of 0 - 20.0 mcg/ml (r=0.9999).

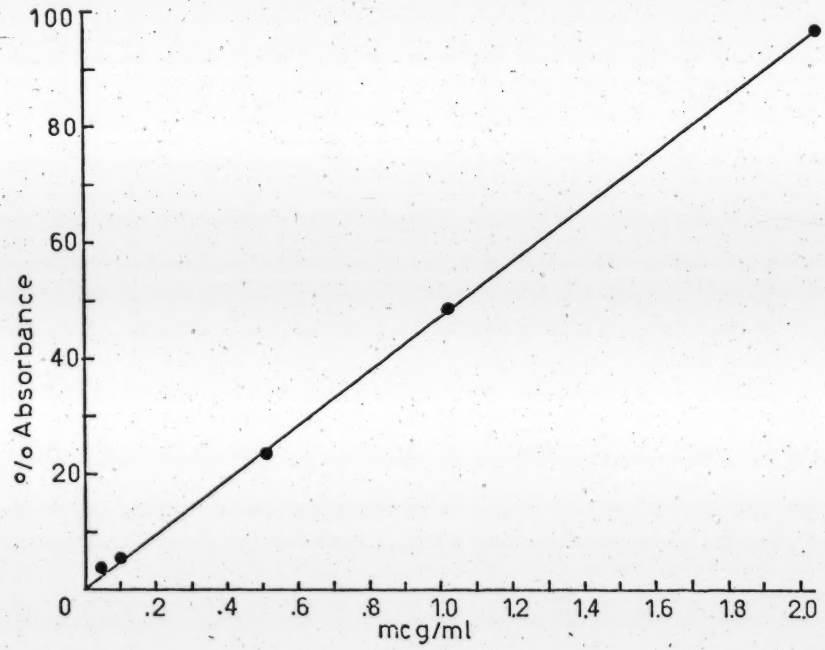


Fig. 8 - Example of Standard Curve of Indomethacin for Plasma Concentration Ranges of 0 - 2.0 mcg/ml (r=0.9998).

2.0 mcg/ml in plasma were also prepared and analyzed; linear correlations (r=0.9998, r=0.9994) were found.

2. Assay Specificity.

Table VII presents the results obtained for indomethacin measurements when different concentrations of the two metabolites DMI and DBI were added to the I solutions. Stability after one and three weeks of storage in the refrigerator 3°C of 100, 10 and 1 mcg/ml standard solutions was tested and the following results were obtained as shown in Table VIIIa. The stability of indomethacin, 1, 10 and 100 mcg/ml, in frozen plasma samples was followed for a three week period; the results obtained are summarized in Table VIIIb. Figures 7 and 8 show sample standard curves of indomethacin in plasma for plasma concentration ranges of 0-20 and 0-2 mcg/ml, respectively.

C. Animal Studies

1. Radiological.

a. Gastrointestinal transit time.

The mean gastrointestinal transit times in Table IXa were obtained from determinations in 5 different monkeys. Table IXb shows the gastrointestinal times obtained on monkeys D and G, where a monkey for each model was followed by both techniques under pre- and post- surgical conditions. In some instances, 20 ml of mineral oil was administered to monkey C (pre- and post- surgery), and to monkey F (pre-surgery), 16 hr prior to the g.i. transit time determinations with either barium sulfate or the radiopaque beads. From these studies (n=5), transit times of 0.75, 0.75, 1.25, 1.0 and 1.25 hr ($\bar{x}$=1.0 hr), were observed. Since the pre-administration of mineral oil to the monkey caused a

TABLE VII. Indomethacin Assay Specificity.

Sample				Concentration of Indomethacin (mcg/ml)		
	Concentration (mcg/ml)			Mean	Standard Deviation	P
No.	I	DMI	DBI	n=3	n=3	
1	-	-	-	0. **	-	-
2	100	-	-	100.0*	-	-
3	10	-	-	10.167	0.565	NS ***
4	1	-	-	0.983	0.116	NS
5	0.1	-	-	0.105	0.012	NS
6	100	100	100	99.333	1.863	NS
7	100	10	10	99.500	1.500	NS
8	100	1	1	100.467	0.674	NS
9	10	100	100	11.333	1.459	NS
10	10	10	10	10.230	1.569	NS
11	10	1	1	9.867	0.729	NS
12	1	100	100	3.633	0.907	P<0.001
13	1	10	10	1.733	0.225	P<0.001
14	1	1	1	1.016	0.145	NS
15	0.1	100	100	3.513	0.934	P<0.001
16	0.1	10	10	0.613	0.058	P<0.001
17	0.1	1	1	0.127	0.127	NS

P=Probability that the two means are members of the same population.
* Stock solution used to adjust 100% reading on the instruments.
** Blank
*** When P>.05

TABLE VIIIa. Stability of Indomethacin Solutions

Concentration (mcg/ml)	One Week Storage			Three Week Storage		
	n	Mean	Standard Deviation	n	Mean	Standard Deviation
100.0	3	98.52	0.71	3	84.33	4.51
10.0	3	9.73	0.16	3	8.07	0.25
1.0	3	0.97	0.03	3	0.84	0.05

TABLE VIIIb. Stability of Indomethacin in Frozen Plasma Samples (*).

Concentration (mcg/ml)	One Week Storage		Two Week Storage		Three Week Storage	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
100.0	107.0	10.4	97.3	3.7	100.6	3.4
10.0	10.4	0.64	9.90	0.50	9.5	0.85
1.0	0.92	0.32	0.84	0.19	1.13	0.25

*: n=4

TABLE IXa. Gastrointestinal Transit Time, (Hrs).

	Pre-Surgery		Post-Surgery			
			Model I		Model II	
	$BaSO_4$ Suspension	Beads	$BaSO_4$ Suspension	Beads	$BaSO_4$ Suspension	Beads
n (Monkey)	3 (D,G,H)	3 (D,F,G)	3 (C,C,D)	3 (C,D,D)	3 (F,G,G)	3 (F,G,G)
Mean	2.50	2.33	2.50	2.67	1.67	2.67
S.D.	0.43	0.58	0.50	0.29	0.29	0.29

TABLE IXb. Gastrointestinal Transit Time (Hrs) in Monkeys D and G.

Model (Monkey)	Pre-Surgery		Post-Surgery	
	$BaSO_4$ Suspension	Beads	$BaSO_4$ Suspension	Beads
I (D)	3.0	2.0	3.0	2.5
II (G)	2.25	2.0	1.5	2.5

marked effect on the transit times noted, such values were excluded from the calculation of the data shown in Table IXa.

Transit time studies with non-disintegrating cores (samples N1 and N2) were performed on two different monkeys prepared only with vascular catheters and gastric cannulae. Gastrointestinal transit times obtained with sample N2 averaged 2.08 hours (n=3, S.D.=0.29). Samples N1 moved very slowly along the monkey's gastrointestinal tract, (monkey H); one capsule remained in the stomach of the animal for at least 6.0 hours and in another instance a capsule was found in the large intestines of the animal ten days after administration.

Radiological studies were carried out so as to follow the emptying pattern from the stomach. One hundred "beads" were administered in a size #0 hard gelatin capsule to the monkey via the gastric cannula; x-ray plates were taken at 15 or 30 minute intervals until the end of the experiments. The results shown in Table X were obtained from two separate studies on the same monkey; in both instances the gelatin capsule had dissolved within 2 minutes after administration.

b. Effectiveness of ileo-cecal blockade and ileo-jejunal connection, (Model I).

In the Model I preparations, the effectiveness of the blockade at the ileocecal level was tested by radiological means. The blockade procedure was always found to effectively prevent the passage of ileal contents to the large bowel. The effectiveness of the ileo-jejunal connection was also tested and it was found that most of the administered barium sulfate suspension passed from ileum to jejunum, even though small amounts of the radiopaque medium passed from jejunum to ileum. The

TABLE X. Gastric Emptying Pattern of Radiopaque Beads in Monkey H.

Time (Hours)	Percent Remaining in Stomach	
	Study 1	Study 2
0	100	100
0.5	100	100
1.0	100	100
1.5	80	100
2.0	10	100
2.5	3	100
2.75	-	99
3.0	0	-
3.25	-	98
3.5	-	96
3.75	-	77
4.25	-	12
4.75	-	5
5.25	-	3
5.75	-	2
6.25	-	0

flow through the latex tubing connecting the UJ and TI cannulae to each other was found to be very slow; the radiopaque medium mixed with intestinal contents required about 4 hours to travel the 13 cm of latex tubing separating TI from UJ. To make Model I more efficient it was considered best to collect the intestinal fluids at TI by means of an attached latex tubing and readminister them at UJ by means of a foley catheter 20 Fr. and a syringe.

2. Indomethacin.

a. Intravenous solution.

Indomethacin solutions were administered intravenously to 8 different monkeys at doses of 5 mg/kg body weight. Figure 9 is a typical semilogarithmic plasma concentration versus time plot of indomethacin in the monkey after i.v. administration. The plasma levels versus time data was fit to a two-compartment model assuming that the later points involved enterohepatic recycling of the drug (5). The parameters V_1, k_{21}, α and β shown in Table XI were estimated by means of the nonlinear least squares computer program, NONLIN (6). The rest of the parameters shown were calculated from these computer estimates.

The Loo-Riegelman method (7) was applied to the data obtained from all studies in order to estimate the total amount of indomethacin reaching the systemic circulation. To calculate the concentration of drug in the second compartment Eq.3 was utilized:

$$(C_2)_{tn} = (C_1)_{tn-1} \cdot \frac{k_{12}}{k_{21}} \cdot \left(1 - e^{-k_{21}\Delta t}\right) + (C_2)_{tn-1} \cdot e^{-k_{21}\Delta t} + \frac{k_{12}}{2} \cdot \Delta C_1 \cdot \Delta t \qquad (Eq.\ 3)$$

where $C_2 = A_2 / V_1$.

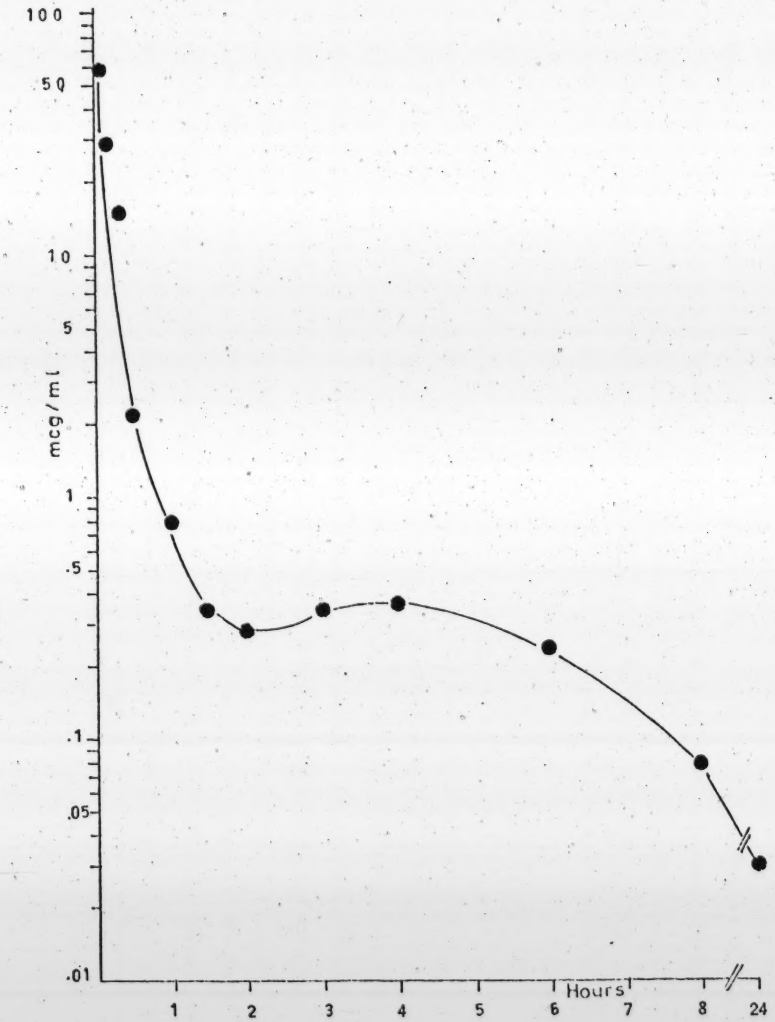
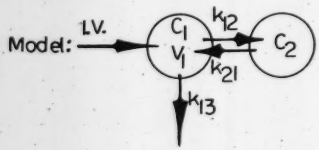


Fig. 9 - Typical Plasma Concentration versus Time Plot after I.V. Administration of Indomethacin to Monkey D. Dose=5 mg/kg.

TABLE XI. Summary of Pharmacokinetic Parameters of Indomethacin Disposition.

Monkey	A	B	C	D	E	F	G	I	Mean	S.D.
B. W. (Kg)	7.2	7.0	5.4	5.4	8.6	9.7	6.2	5.0	6.8	1.7
Dose (mg)	36	35	27	27	43	50	31	25	-	-
n For Nonlin Fit	9	9	9	7	7	9	15	8	-	-
V_1 (L)	0.385	0.401	0.812	0.214	0.600	0.958	0.440	0.343	0.519	0.252
k_{21} (hr^{-1})	0.492	0.515	0.713	0.693	0.807	0.805	0.449	0.773	0.656	0.148
α (hr^{-1})	4.340	7.520	3.558	8.457	6.010	5.710	5.076	10.487	6.395	2.296
β (hr^{-1})	0.372	0.449	0.536	0.632	0.615	0.631	0.359	0.646	0.530	0.121
k_{13} (hr^{-1})	3.281	6.566	2.676	7.707	4.578	4.477	4.060	8.761	5.263	2.173
k_{12} (hr^{-1})	0.939	0.976	0.705	0.689	1.240	1.060	0.926	1.598	1.017	0.295
$k_{13}V_1$ (L/hr)	1.263	2.633	2.173	1.649	2.747	4.289	1.786	3.005	2.443	0.995
$k_{13}V_1$/B.W.(L/Kg-hr)	0.175	0.376	0.402	0.306	0.319	0.442	0.288	0.601	0.364	0.126
V_1/B.W. (L/Kg)	0.053	0.057	0.150	0.040	0.070	0.099	0.071	0.069	0.076	0.034



$$\alpha + \beta = k_{12} + k_{21} + k_{13}$$

$$\alpha \cdot \beta = k_{21} \cdot k_{13}$$

The total amount of the drug reaching the systemic circulation was estimated by means of Eq. 4.

$$(A_1)_{tn} / V_1 = (C_1)_{tn} + (C_2)_{tn} + k_{13} \int_0^{tn} C_1 \, dt \qquad (Eq. 4)$$

Figure 10 illustrates the application of this procedure to i.v. indomethacin data. Tables 2A and 2B (see Appendix) show sample tabulations for the estimation of $(C_2)_{tn}$ and $(A_1)_{tn}/V_1$ by the Loo-Riegleman method, after i.v. administration of indomethacin to monkey D.

b. Oral solution.

Figures 11 and 12 are semilogarithmic plasma concentration versus time plots exemplifying the data obtained after the administration of indomethacin solutions to the monkeys via the gastric cannula. Figure 11 depicts the composite results of three different experiments conducted on monkey B, while Figure 12 shows the results from a single experiment on monkey C. The plasma concentration versus time data obtained was treated according to Eqs. 3 and 4, assuming the disposition parameters (V_1, k_{21}, α and β) remained the same as after i.v. administration. Figures 13 and 14 show the cumulative amount of indomethacin reaching the systemic circulation versus time plots obtained for monkeys B and C. Tables 2C and 2D (see Appendix) summarize the estimation of the amount of drug reaching the systemic circulation (Loo-Riegelman method) after oral administration of indomethacin solutions to monkeys B and C, respectively.

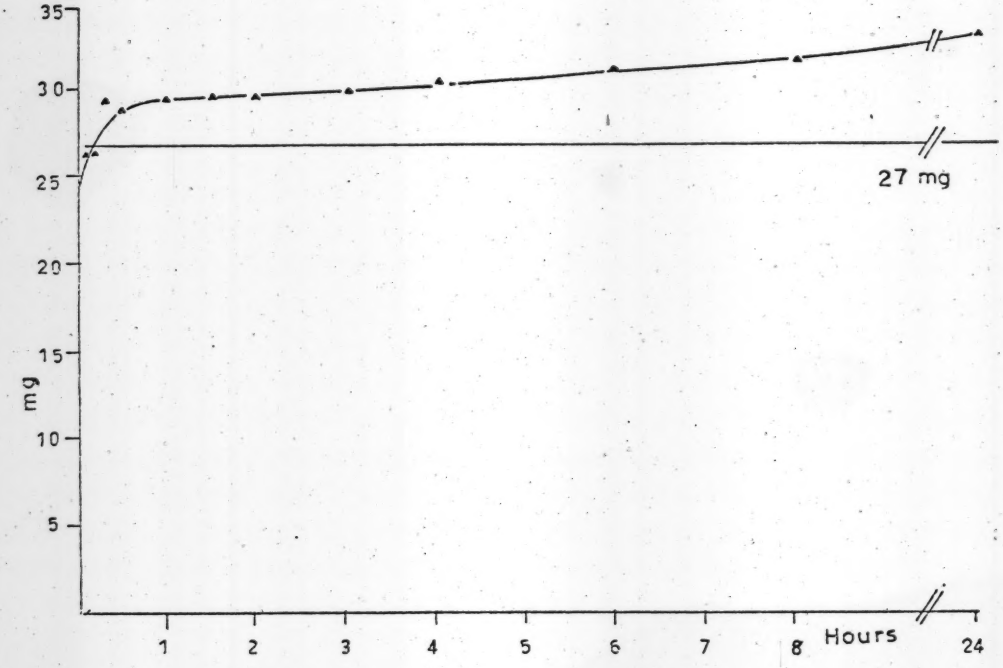


Fig. 10 - Cumulative Amount Absorbed versus Time Plot after Indomethacin I.V. Administration to Monkey D.

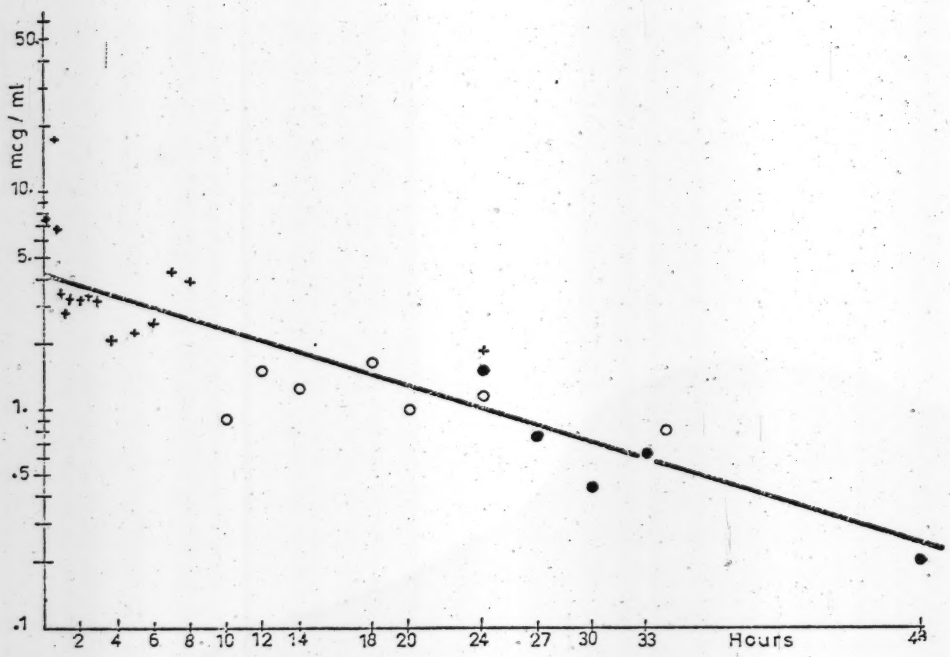


Fig. 11 - Indomethacin Plasma Concentration versus Time Profile in Monkey B after Oral Administration of the Drug in Solution (90 mg/ 50 ml Sodium Phosphate Buffer pH=8). The Different Symbols Represent Studies Conducted at Different Dates.

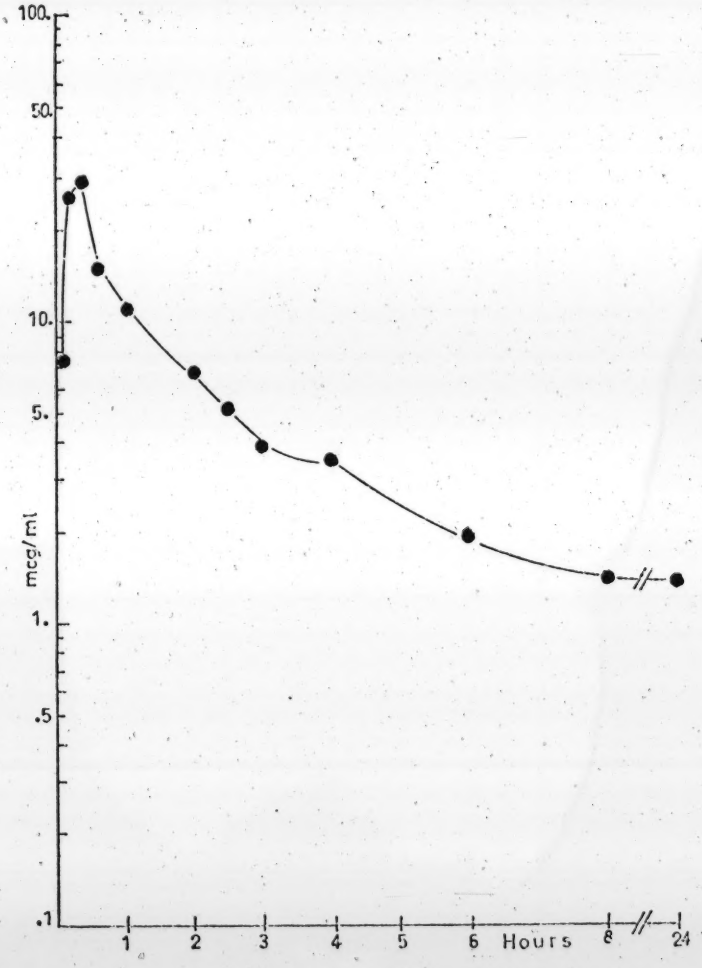


Fig. 12 - Indomethacin Plasma Concentration versus Time Profile in Monkey C after Oral Administration of the Drug in Solution (100 mg/ 25 ml Sodium Phosphate Buffer pH=8).

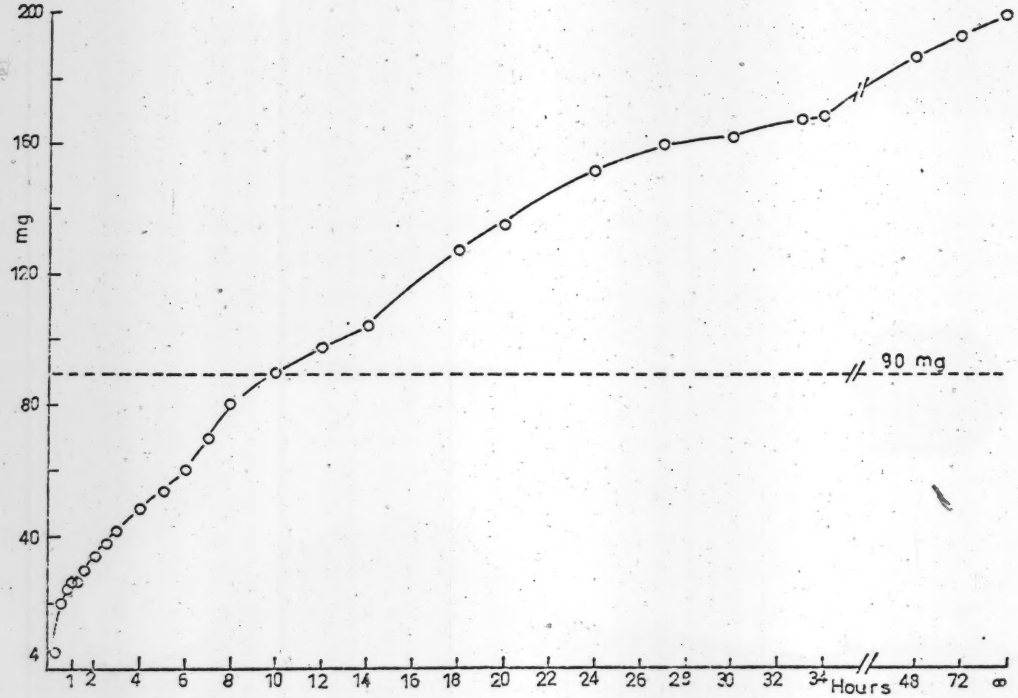


Fig. 13 - Cumulative Amount of Indomethacin Reaching the Systemic Circulation (Loo-Riegelman Method) after Oral Administration of the Drug in Solution to Monkey B (Dose=100 mg). Calculated Using Data Presented in Fig. 11.

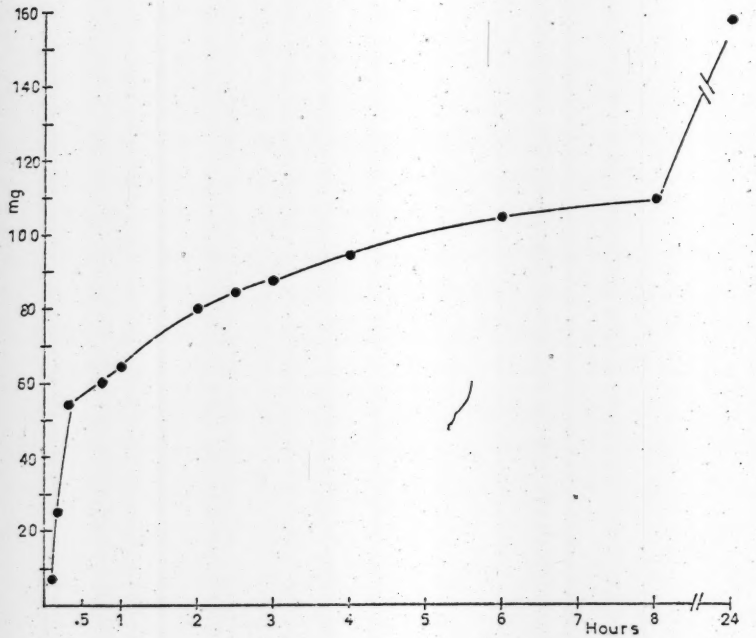


Fig. 14 - Cumulative Amount Reaching the Systemic Circulation (Loo-Riegelman Method) versus Time Profile after Oral Administration of Indomethacin in Solution to Monkey C (Dose =100 mg). Calculated using Data Presented in Fig. 12.

c. Oral conventional (In-line) capsules.

In-line indomethacin capsules as control samples were administered orally to animals prepared as per Model I (monkey D) and Model II (monkey G). Semilogarithmic plots of the plasma concentration versus time course of indomethacin for these studies are shown in Fig. 15. The corresponding amount of drug absorbed versus time plots are shown in Fig. 16. Tables 2E and 2F (see Appendix) summarize the estimation of the cumulative amount reaching the systemic circulation (Loo-Riegelman method) after oral administration of in-line indomethacin capsules to monkeys D and G, respectively.

d. Oral controlled release capsules.

Figure 17 shows the semilogarithmic plasma concentration profiles of the controlled release preparations P1 and G1 when tested in Model I (monkey D). Correspondingly, Fig. 18 shows the amount absorbed versus time plots. Similarly, Fig. 19 shows the semilogarithmic plasma concentration versus time profiles of the controlled release preparations P2 and P3. Figure 20 shows a similar profile for preparation G2 when tested in Model II, (monkey G). In Figs. 21 and 22 the corresponding cumulative amounts reaching the systemic circulation versus time plots can be seen. Tables 2G and 2H (see Appendix) summarize the application of the Loo-Riegelman method to the Cp-vs-t data obtained after the oral administration of the controlled release preparations P1 and G2 to monkey D, respectively. Tables 2I, 2J and 2K of the Appendix show the corresponding data for the controlled release preparations P2, P3 and G2 in monkey G, respectively.

The percentages of the administered dose reaching the systemic

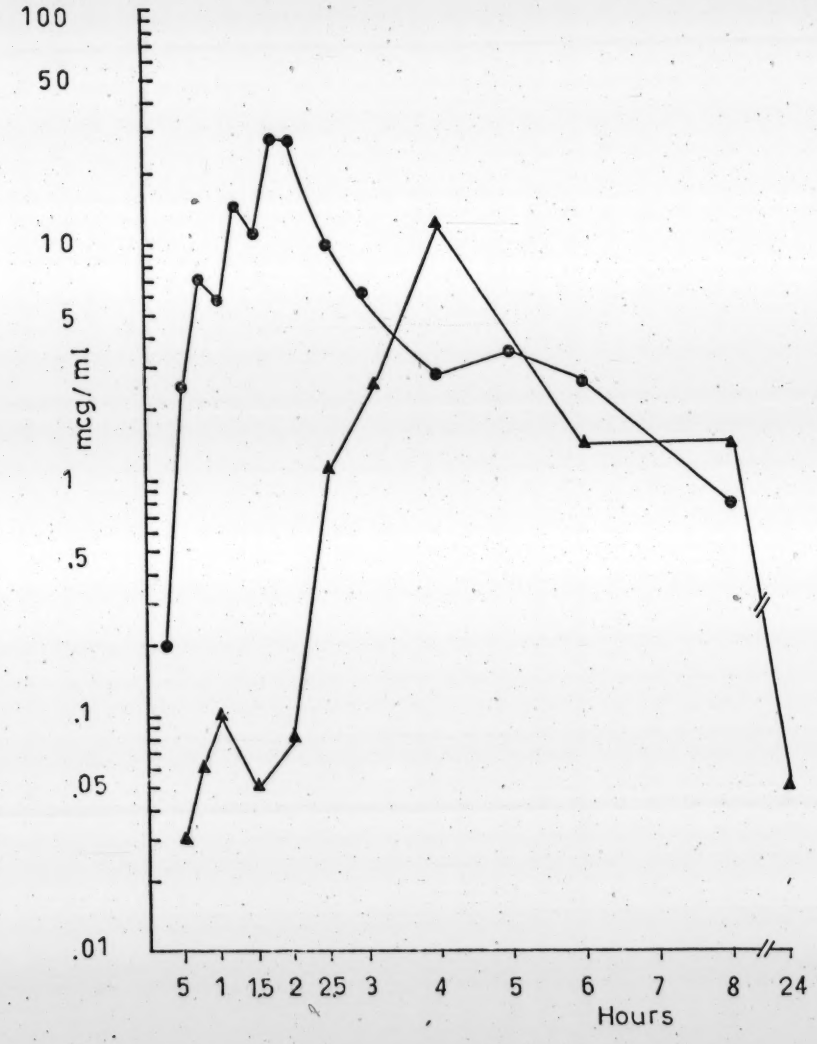


Fig. 15 - Semilogarithmic Plasma Concentration versus Time Plot after Oral Administration of In-line Indomethacin Capsules to: -▲- Monkey D (Dose=100 mg), and -●- Monkey G (Dose=75 mg).

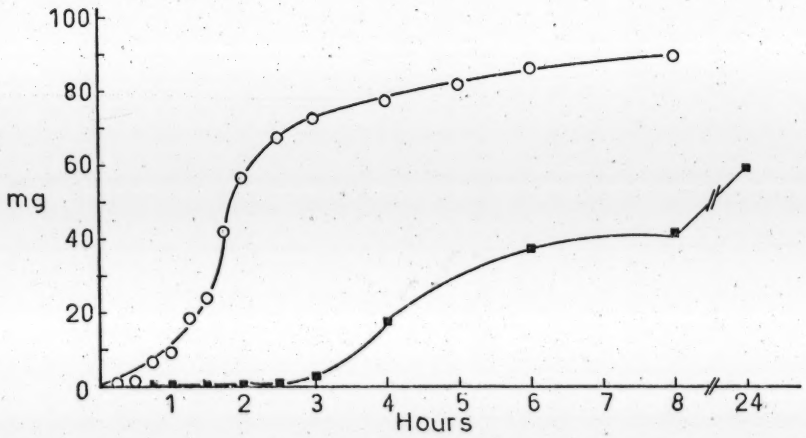


Fig. 16 - Cumulative Amount of Drug Reaching the Systemic Circulation versus Time Profile after Oral Administration of In-line Indomethacin Capsules to: —■— Monkey D (Dose=100 mg), and —○— Monkey G (Dose=75 mg). Calculated from Data Presented in Fig. 15.

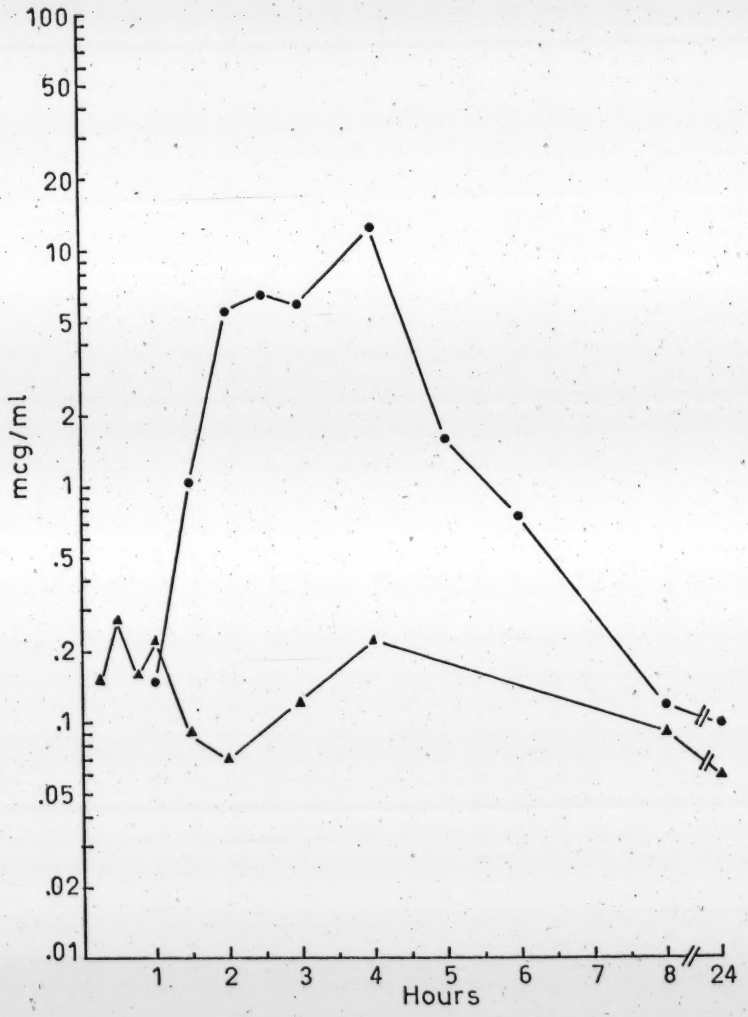


Fig. 17 - Semilogarithmic Plasma Concentration versus Time Plot of Indomethacin in Monkey D after Oral Administration of Controlled Release Preparations: -▲- Pl, and -●- Gl. (Dose=75 mg).

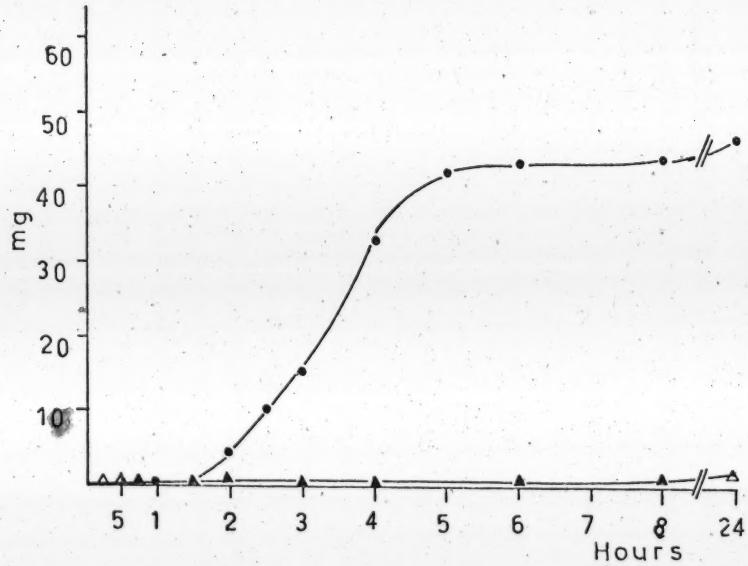


Fig. 18 - Cumulative Amount of Drug Reaching the Systemic Circulation versus Time Profile (Loo-Riegelman Method) in Monkey D after Oral Administration of Indomethacin Controlled Release Preparations: -▲- P1, and -●- G1 (Dose=75 mg). Calculated from Data Presented in Fig. 17.

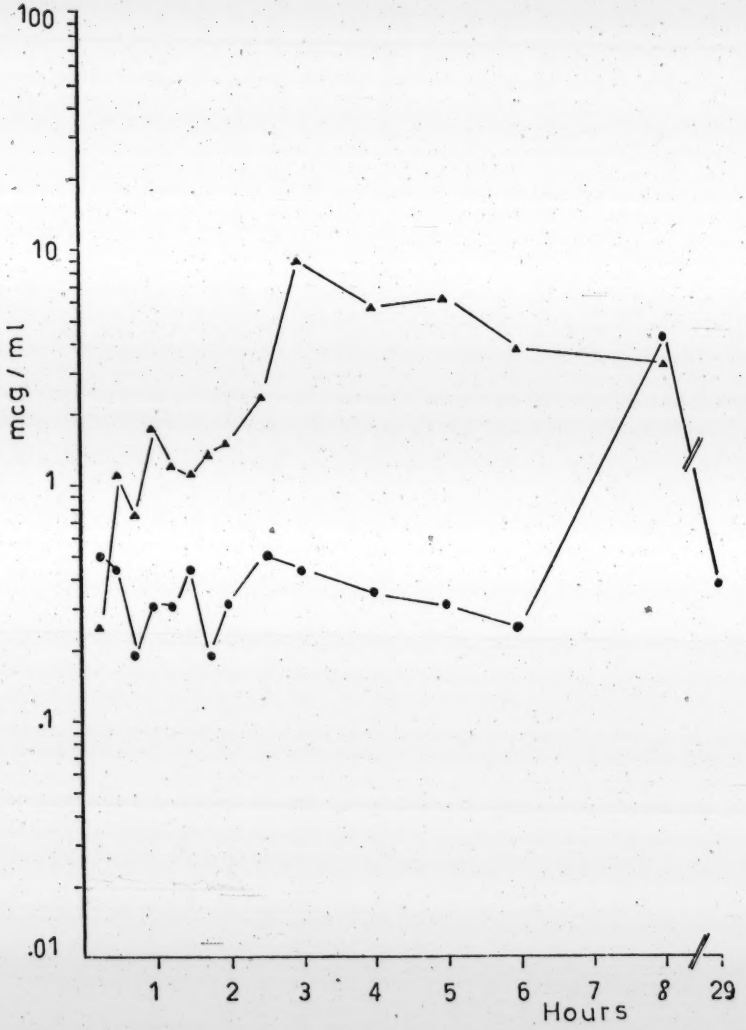


Fig. 19 - Semilogarithmic Plasma Concentration versus Time Plot after Oral Administration of Indomethacin Controlled Release Preparations -●- P2, and -▲- P3 to Monkey G (Dose=75mg).

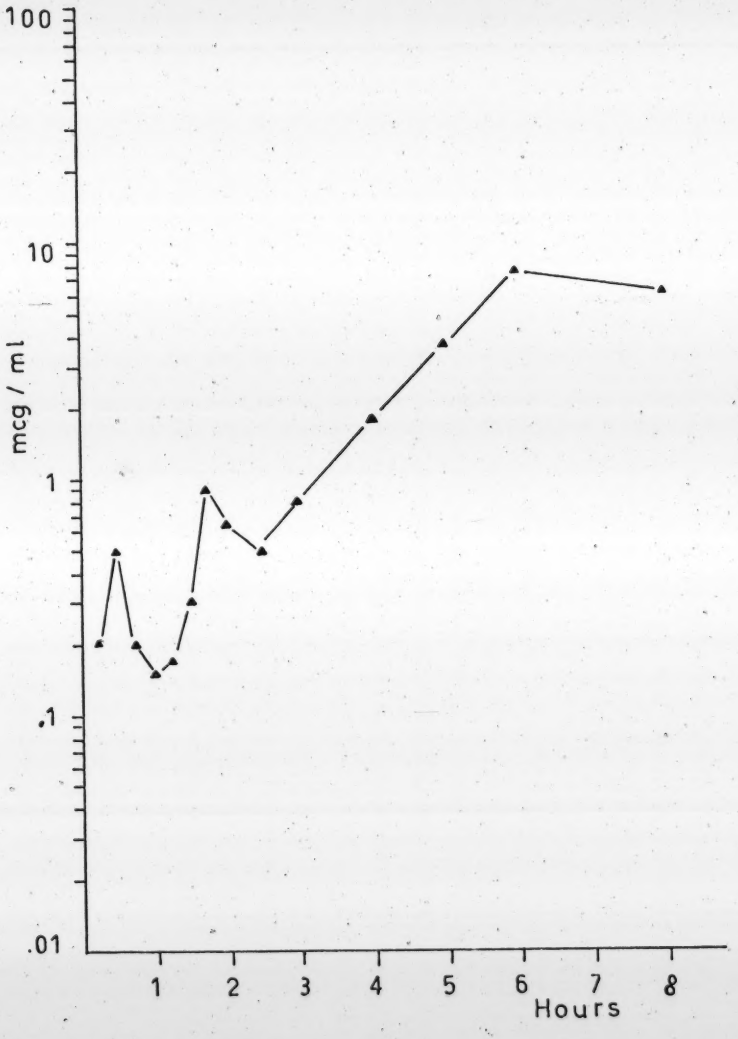


Fig. 20 - Semilogarithmic Plasma Concentration versus Time Plot after Oral Administration of Indomethacin Controlled Release Preparation G2 to Monkey G (Dose=75 mg).

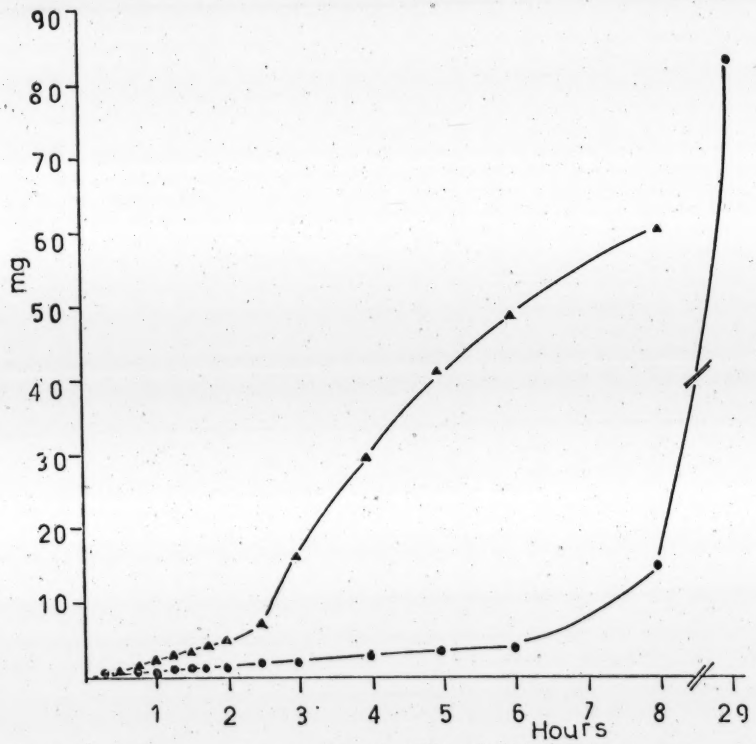


Fig. 21 - Cumulative Amount of Drug Reaching the Systemic Circulation versus Time Profile after Oral Administration of Indomethacin Controlled Release Preparations: -●- P2, and -▲- P3 to Monkey G (Dose=75 mg). Calculated from Data Presented in Fig. 19.

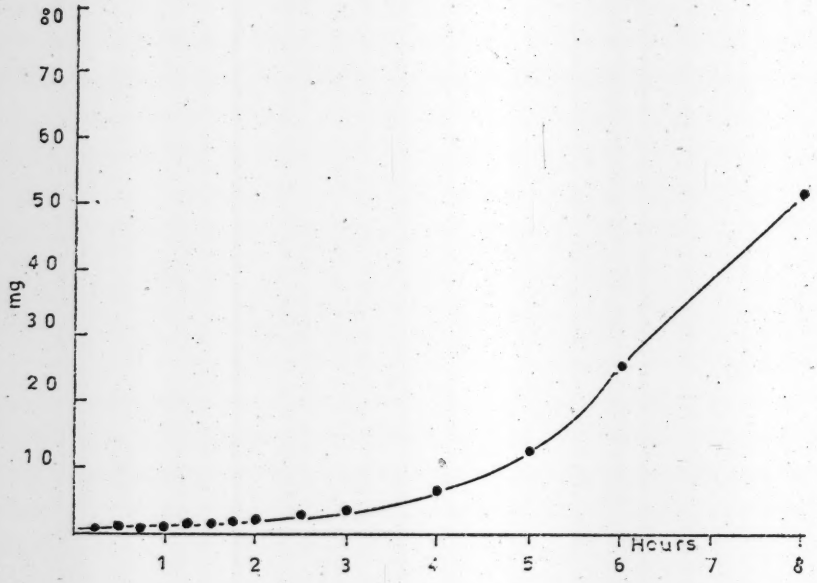


Fig. 22 - Cumulative Amount of Drug Reaching the Systemic Circulation after the Oral Administration of Indomethacin Controlled Release Preparation G2 to Monkey G (Dose=75 mg). Calculated from Data Presented in Fig. 20.

circulation after the four different types of studies conducted, are presented in Table XII.

In addition, one study was conducted in which 90 mg of indomethacin in 25 ml of sodium phosphate buffer pH=8, was administered to monkey B via the TI cannula which was then connected to UJ. Passage to the large bowel was prevented. The following plasma levels were found:

t (hrs) :	0.	0.5	2.	3.	4.	6.	24.
Cp (mcg/ml):	0.	125.	51.5	50.	2.5	68.	36.

At the 6th hour post-administration, the contents in the connecting tubing were collected and sampled; the total amount of indomethacin remaining in the tubing was determined and found to be 8 mg. This quantity was not readministered to the monkey. Thus, the total administered dose = 82 mg.

In another study four in-line indomethacin capsules (25 mg each) were placed in the ileo-jejunal connection in monkey B; passage to the large bowel was prevented. The following results were obtained:

t (hrs) :	0.	0.167	0.5	1.	2.	24.
Cp (mcg/ml):	0.	0.1	3.2	3.9	0.15	0.05

At the second hour post-administration, the contents in the connecting tubing were collected and sampled; the total amount of indomethacin remaining in the tubing was determined and found to be 20 mg. This quantity was not readministered to the monkey. Total dose administered = 80 mg.

Table XII. Summary of the Percent of Dose Reaching the Systemic Circulation After Administration of Indomethacin to 6 Rhesus Monkeys.

	I.V. Solution		Oral Solution		Oral In-line Capsules		Oral C.R. P1		Oral C.R. G1		Oral C.R. G2		Oral C.R. P2		Oral C.R. P3	
	Hours	%	Hours	%	Hours	%	Hours	%	Hours	%	Hours	%	Hours	%	Hours	%
A	8 24 30	140.28 159.42 166.67	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	1.5	108.22	8 24 72	81.43 151.87 215.51	-	-	-	-	-	-	-	-	-	-	-	-
C	8 28	123.48 143.67	8 24 28	121.33 157.74 169.77	-	-	-	-	-	-	-	-	-	-	-	-
D	8 24	118.07 123.57	8 24	55.81 99.70	8 24	41.51 58.78	8 24	1.97 4.64	8 24 30	59.28 62.86 64.66	-	-	-	-	-	-
E	8 24	122.47 128.93	-	-	-	-	-	-	-	-	8 12	180.0 200.11	-	-	-	-
F	8 -	118.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-
G	6 28	106.13 119.26	-	-	8 24	118.99 119.37	-	-	-	-	8 -	67.84 -	8 29	20.05 111.13	8 -	80.52 -
I	8 24	170.20 224.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Generated Data	10 ∞	108.22 108.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-

D. References

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V. DISCUSSION

The results obtained in the development and testing of an animal model for the evaluation of oral controlled release preparations, will be discussed now. The sequence followed is similar to the general framework utilized in the preceding chapters -- animal set-up, indomethacin assay and animal studies.

A. Animal Set-up

1. Surgical Preparation.

Before the initial surgery was performed on a new monkey an adaptation period to the restraining chair of at least 2 days was allowed. Thus, when needed, any adjustments to the restraining chair for a particular monkey could be accomplished before the animal was surgically prepared.

In all instances, the vascular catheterization was the first operation performed. The implanted catheters allowed us to carry out intravenous studies and thus to determine the disposition parameters of indomethacin in the restrained unanesthetized rhesus monkey. Subsequently, the gastric cannula was implanted. It has been observed in this laboratory that when properly cared for, the monkey prepared with vascular catheters and a gastric cannula survives and remains functional for years (1 , 2). If the vascular catheters implanted on the left side become clogged, they can easily be removed from the immobilized animal (20 mg/kg i.m. dose of ketamine hydrochloride) and new catheters may be implanted into the right femoral vessels; if these catheters also become non-functional, still another pair might be implanted in the jugular vein and carotid artery of the animal. Monkey A was prepared following such a sequence. Cannula G provided a means of

instilling a drug solution or introducing an intact solid dosage form directly into the stomach, as if orally administered.

In the normal gastrointestinal physiology of the monkey, the terminal and upper-jejunum portions are adjacent to each other due to the convolutions of the small intestine. By means of an appropriate by-pass between these two portions and blockade of the ileocecal valve, the contents of the lower ileum could be shunted into the upper jejunum and recycled through the small intestine.

A surgical technique was developed for the implantation of cannulae B, UJ and TI as described previously. Once the technique had been developed, it became possible to conduct the implantation of all cannulae: B, UJ, TI and G, at the same time.

2. Model I.

One monkey (F), out of four Model I preparations did not recuperate from the intestinal cannulae implantation (Table I). In that particular case, a localized infection at the terminal ileal cannulae site was the reason for the failure of the animal to recover from surgery.

In three out of four multicannular monkeys (i.e., not considering Monkey F), the upper terminal-ileal cannula was extruded, on an average, 46 days after implantation. Such reaction to foreign bodies occurs uniformly in animals and man.

3. Model II.

Based on the results obtained with Model I preparations, specifically the terminal ileal cannula extrusion reaction, it was thought that the patency of the model could be improved by the substitution of an ileostomy for cannula TI and thus eliminating the need for cannula B since the ileostomy would cause a permanent closure of the large bowel.

In this fashion, the intestinal contents could be collected in the ileostomy bag and readministered to the monkey through UJ, thus complying with model requirement three, that is, that the model should enable the dosage form to be in contact with the absorbing mucosa for a period of at least 8 to 12 hours.

As far as we are aware, this is the first time that ileostomy in monkeys has been described.

A recent or unadapted ileostomy may discharge profuse volumes of water and large concentrations of electrolytes, such as sodium, chloride, potassium and bicarbonate into the external environment. Ileal fluid contains a significant quantity of potassium and its loss is usually associated with clinical signs and symptoms of hypokalemia. The "diarrhea" decreases as the ileostomy matures and from an ileostomy output of one liter or more, subsides to a volume of 400-600 ml/day of a semisolid discharge containing less than 120 mEq/L of sodium. Wright and Tilson had pointed out that for man the sodium concentration of the discharge usually falls in the presence of a negative sodium balance, but does not rise above an average of 120 mEq/L, because of the ability of the mucosa of the normal distal ileum to produce and maintain a sodium concentration in the ileal contents significantly lower than the normal plasma sodium concentration (3). Comparison of sodium levels in serum, Table III, and in ileal fluid, Table V, for monkey J show that similar findings are observed in the ileostomized monkey. It can also be seen that the loss of sodium in the ileal fluid is disproportionately large compared with that of chloride which, as mentioned by Wilkinson (4), leads to acidosis. The acute, profuse, external discharge of ileal fluid causes losses

of water, sodium, chloride, bicarbonate and potassium. As a result of water loss, the blood volume contracts and the hematocrit rises. A relative increase in the concentration of carbonic acid and an actual increase in the number of hydrogen ions occur in the blood and cause metabolic acidosis. The net result of these changes is base-losing metabolic acidosis and isotonic dehydration. The average reported pH of monkey blood ranges from 7.34 to 7.5 depending upon the sampling technique utilized (5). Forsyth and collaborators utilizing a monkey system with vascular catheters similar to ours, have reported arterial blood pH's averaging from 7.39 to 7.5 (6), 7.4 (7), and from 7.36 to 7.42 (8). In only one instance, Monkey E, and 14 days after the surgery, the arterial blood pH of an ileostomized monkey was determined. The value found, 7.37, lies among the lower normal values quoted. The hematocrit value found on the same date was 12.1% higher than that obtained before the surgery.

Losses of sodium and potassium have to be replaced by an intravenous infusion of saline or lactated Ringer solution with added potassium chloride (0.2g/100ml, 27 mEq/L of potassium) (4). The serum concentration of sodium is not usually of much help in estimating how much sodium should be administered. Close and careful observation of the effect of treatment on the animal is probably the best means of judging success. When signs of sodium and water depletion appear, treatment must be thorough and rapid and its accuracy is improved if all the fluid lost from the ileostomy, as well as all urine are collected and the volume, sodium and potassium contents measured.

We observed changes in the eating habits of the ileostomized monkeys.

In the case of monkey J, the animal ingested only 10 Purina Monkey Chow^{R} "briquettes" during the first week after the surgery; his nutrition was supplemented with 100 ml/day of Ambex^{R} solution. Later, his food intake increased to 8 "briquettes"/day, still much lower than his 20-30 "briquettes"/day pre-surgery intake. A 26.7% reduction in body weight was recorded from the date of surgery to the date of death; the apparent reason for his death was malnutrition. In another instance, monkey E, a decrease in eating habits was also observed, although not as marked as in monkey J. Monkey E's serum electrolyte levels were lower than the pre-surgical values and his arterial blood pH was normal. Blood cells, hematocrit and hemoglobin values were respectively unchanged, 19.2% and 12.1% higher than the values found prior to the surgical preparation of the monkey. All values however, were still within the normal range as reported by Krise and Wald (9). A body weight decrease was observed. Monkey E died 24 days after the surgery. A necropsy was performed and upon visual examination, lungs, heart, liver, stomach and intestines appeared normal. Again, the death of the animal was assumed to have been caused by malnutrition.

As can be seen from Table XII only monkey G was practically useful as a model for the evaluation of controlled release formulations, and then only for a limited period of time (44 days). From our overall results with Model II preparations it seems that without further assistance rhesus monkeys of this size (7.0 Kg average body weight), in general do not adjust successfully to the changes brought about by the ileostomy, i.e., electrolyte depletion, dehydration, metabolic acidosis, alterations in the intestinal microflora and in the

intestinal fluid composition. Since nutrient absorption takes place at a level higher than the ileostomy, and electrolyte, water and bile salt metabolism are normal (3), we believe that many of the problematic alterations could be overcome by the continous recirculation of the ileostomy output back into the monkey's intestinal tract. This alternative could best be accomplished by the collection of the ileal fluids in the ileostomy bag and the continuous readministration of these to the monkey via the UJ cannula using a peristaltic pump.

B. Quantification of Indomethacin

The modified assay developed by Drs. Wynosky, Porter and Grabowski has been reported to be specific for unchanged indomethacin, without interference from indomethacin metabolites (10, 11). Galeazzi and Benet have found an average daily coefficient of variation (C.V.) of 3.9% for standards of 0.5 to 20 mcg/ml, which is in close agreement with the 2.83% obtained by us for standards of 1 to 100 mcg/ml. For the 0.1 mcg/ml standard we obtained C.V.'s within the range of 11 to 12%.

Indomethacin concentrations determined in the presence of various concentrations of its metabolites DMI and DBI (see Table VII) were compared with the measurements obtained for indomethacin alone. The corresponding means were tested for significance of difference utilizing a two-tailed t test (12, 13). The differences obtained in indomethacin concentration measurements proved to be not significant at the 0.05 level, with the exception of samples No. 12, 13, 15 and 16, as shown in Table VII. It is worth noting however, that Duggan et al. (14)

have shown that after either i.v. (25 mg) or oral (50 mg) administration of indomethacin to human subjects, the plasma concentrations of the unchanged drug, from 0 to 8 hrs, are higher than those of both metabolites. The lowest plasma concentration of I shown by Kwan et al. (11) after i.v. (25 mg), oral or rectal (50 mg) single dosing is 0.08 mcg/ml, while the mean plasma concentrations of DMI never exceed 0.1 mcg/ml. In addition, these authors report DBI initial levels, after i.v. dosage of I, between 0.1 and 0.2 mcg/ml. After the second hour post-administration, mean DBI concentrations never exceed 0.04 mcg/ml. Yesair et al. (15) after administration of ^{14}C-indomethacin to rhesus monkeys have reported that in the blood samples from 0.33 to 6 hrs post-administration 85% of the extracted radioactivity was indomethacin. From our studies, we have found mean I plasma concentrations at the 8th hour of 0.81 (S.D.=1.34) and 2.95 (S.D.=2.0) mcg/ml after i.v. and oral dosing, respectively. Since at the highest possible ratio of I: DMI: DBI, (1:1:1) no interference was detected, it can therefore be said that at the plasma concentrations usually found after i.v. or oral administration of indomethacin, the metabolites DMI and DBI do not interfere with the quantification of indomethacin by the method of Wynosky, Porter and Grabowski. The stability of standard solutions of indomethacin in sodium phosphate buffer pH=8 was tested after one and three week storage in the refrigerator (3°C) and the difference in the averages were found to be not significant and significant at the 0.05 significance level, respectively.

Because of the decreasing stability of indomethacin solutions with time (Table VIIIa), indomethacin solutions utilized in this work were

prepared either the same day or one day before the assay would be carried out. In our studies, the collected samples were immediately frozen and kept at -15°C until assayed. The stability of indomethacin in frozen plasma samples was also tested. The variance of the data obtained from these studies was greater than that for the stability of indomethacin standard solutions (see Tables VIIIa and VIIIb). The difference between the standard deviations observed could be a reflection of the fact that data from frozen samples is being compared to data obtained from standard solutions. In addition, the frozen plasma analyses reported in Table VIIIb were carried out at a later time by a second investigator. However, it is apparent from the data in Table VIIIb that there is no significant change in indomethacin plasma levels due to freezing for up to 3 weeks. In practice, most plasma samples were analyzed within 1 week of collection and in no case were samples frozen for more than two weeks.

C. Animal Studies

1. Radiological.

a. Gastrointestinal transit time.

In our studies we observed that both the barium suspension and the beads-saline suspension would usually exit from the stomach between 5 to 30 minutes after administration; on the other hand, when beads were given in a gelatin capsule (no water or saline administered at the same time), 96% of the beads remained in the gastric cavity for as long as 3.5 hours, for fasted animals. The values shown in Tables IXa and IXb represent transit times measured as the time required for the passage of the majority of the administered radiopaque material from

the stomach to the cecum, while most of the data in humans described earlier represents the time required for the appearance of barium sulfate at the cecum. From our experiences in fasted rhesus monkeys, we observed that the appearance at the cecum of either the barium sulfate suspension or the beads usually took place around the first hour post-administration and that approximately two additional hours elapsed before the majority of the radiopaque material reached the cecum. We believe that our figures give a more realistic estimate of the movement along the small intestine of the monkey than the recording of just the "appearance at the cecum" time. Our studies have shown that the gastrointestinal transit time in the monkey is considerably faster than that in man. Based on our findings, then, it is clear that the small intestine transit time difference may be one of the prime factors to consider if the evaluation of oral controlled release dosage forms in monkeys is to be extrapolated to man. Therefore, in order to make the g.i. transit time in the monkey comparable to that in man, we considered ways to modify and control it. Thus, Model I and Model II were developed (see Chapter III), where the passage to the large bowel was prevented and the contents of the small intestine were collected at the terminal ileum and readministered at the upper jejunum.

Radiological studies were conducted to determine if the extensive surgery performed to achieve Model I and Model II preparations had affected the gastrointestinal motility as reflected by the g.i. transit time. The post-surgery values found with either the barium sulfate suspension or the radiopaque beads for both models, shown in Tables IXa and IXb, were not significantly different at the 0.05 significance level when compared to those obtained before surgery.

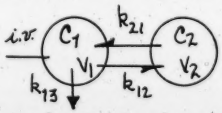
2. Indomethacin.

a. Intravenous studies.

The plasma levels obtained after i.v. administration of indomethacin to the monkeys at dosages of 5 mg/kg, were in the same range as those reported by Yesair et al. (15). As stated in Chapter IV, the Loo-Riegelman method for the estimation of the cumulative amount of drug "absorbed" was applied to the plasma concentration time data obtained after i.v. administration of indomethacin to the monkeys. The correctness of the procedure was tested by the application of the same method to plasma concentration time data generated by means of Eq. 5:

$$(C_1)_{t_n} = \left(\frac{k_{21}-\alpha}{\beta-\alpha}\right)\frac{D}{V_1}\cdot e^{-\alpha t} + \left(\frac{k_{21}-\beta}{\alpha-\beta}\right)\frac{D}{V_1}\cdot e^{-\beta t} \qquad (Eq.5).$$

The assumed model was:



The dose (D=36.0 mg), volume of compartment 1 (V_1=0.973L), and rate constants (α=1.730 hr^{-1}, β=0.514 hr^{-1} and k_{21}=0.645 hr^{-1}) were arbitrarily chosen. The remainder of the rate constants (k_{12}=0.220 hr^{-1} and k_{13}=1.379 hr^{-1}) were calculated by means of Eqs. 6 and 7.

$$k_{12} = (\alpha+\beta)-(k_{13}+k_{21}) \qquad (Eq.\ 6).$$

$$k_{13} = \frac{\alpha\cdot\beta}{k_{21}} \qquad (Eq.\ 7).$$

The calculated cumulative amount of drug reaching the general circulation overestimated the true value by 8%. It should be remembered that the third term in Eq. 3 is the result of an approximation using a

two-term Taylor expansion. Two different approaches were also utilized in estimating the cumulative amount absorbed for this generated data. Firstly, the complete equation of the Loo-Riegelman method (16) was used to estimate $(C_2)_{tn}$, and secondly, $(C_2)_{tn}$ values were estimated by means of a logarithmic expression first presented by Loo (17), as described by Till, Benet and Kwan (18). Since the calculated cumulative amount of drug "absorbed" by these methods also overestimated the true value by 8%, the error was attributed to an intermediate and common step for all three methods, i.e., the trapezoidal rule for the estimation of the area under the plasma concentration versus time curve (AUC). This error could be reduced by increasing the number of samples collected. It was then decided to continue utilizing the Loo-Riegelman method for the analysis of the data obtained from oral studies.

Enterohepatic recycling of indomethacin in the rat was first suggested by Brodie et al.(19). Subsequently, several investigators (11,14,15) have evaluated indomethacin enterohepatic circulation not only in several other animal species but also in man. Kwan et al. (11), reported that approximately 50% of an intravenous dose of indomethacin in man undergoes enterohepatic circulation. Thus, the bioavailability of indomethacin to the systemic circulation may exceed the administered dose. Discontinuities in the plasma concentration time curve apparently due to enterohepatic recycling of indomethacin in the monkey can be clearly seen in Figures 9 and 11. As shown in Figure 10 and Table XII, the total amount of drug reaching the systemic circulation after intravenous dosage was always greater than the administered dose.

In 6 monkeys, the extent of such recycling during the first 8 hours post-administration amounts to 32.08% (S.D.=20.40), and for up to 24 hours to 49.82% (S.D.=39.53). The latter estimate agrees closely with that reported by Kwan et al. (11) in man.

b. Oral studies.

Our studies show that indomethacin is rapidly absorbed when given orally as a solution. Values of t_{max}, time to reach maximum plasma level, ranging from 0.133 to 0.75 hours were observed. The t_{max} for in-line indomethacin capsules obtained in Model I and Model II preparations were 4 and 1.75 hrs., respectively. In addition, our studies in monkey B in which either indomethacin solution or in-line capsules were placed in the ileo-jejunal connection, show that the drug is rapidly absorbed at the terminal ileum and/or the upper-jejunum.

Estimates of total AUC corrected by administered dose and body weight, percent of dose "absorbed" (Loo-Riegelman method), and relative bioavailability for the dosage forms studied are shown in Table XIII. Unfortunately, because of the problematic situations encountered in the maintenance of Models I and II, it was not possible to perform additional studies with preparations G1, P1, P2 and P3; consequently we are forced to deal mainly with comparisons of small numbers of studies and their limitations since in some instances great variability in the drug plasma levels was observed, both intra- and inter-subject, for the same treatment.

The enterohepatic recycling of indomethacin in the monkey proved to be a complicating factor in the rigorous evaluation of the dosage forms under study. For example, in some particular cases there was

Table XIII. Percent Dose Absorbed Estimates for the Different Dosage Forms Evaluated in the Rhesus Monkey (Model I and/or Model II).

		I.V. Mean (S.D.) n=6	Oral Solutions Mean (S.D.) n=3	In-line Capsules Mean (S.D.) n=2	G2 Mean (S.D.) n=2	G1 n=1	P1 n=1	P2 n=1	P3 n=1
$(AUC_0^\infty)^{1,2} \times 10^3$		4.89 (2.77)	5.38 (.44)	3.08 (1.52)	5.07 (1.86)	2.34	0.22	4.59	3.53
% of Dose Absorbed, (Loo-Riegelman)	Hr. 8	132.08 (20.40)	86.19 (33.02)	80.25 (54.79)	123.95 (79.34)	59.28	1.97	20.05	80.52
	24	149.82 (39.53)	141.70 (36.72)	89.08 (42.84)	157.56 (66.97)	62.86	4.64	111.13[4]	101.57
	∞	162.58 (49.34)	195.83 (40.08)	89.90 (41.68)	157.56 (66.97	71.52	6.45	134.87	101.57
Relative Availability for Experimental Dosage Forms[3], %		...	...	...	n=1 90.45	116.75	11.07	110.66	84.98

1 $AUC_0^\infty = \int_0^\infty C_1\,dt + \frac{C_n}{k_1}$.

2 Normalized by (Dose/Body Weight).

3 Relative Availability $= \frac{(AUC_0^\infty)_x}{(AUC_0^\infty)_{\text{In-line caps.}}} \times 100$.

4 29 hr data.

almost no difference between the percent of dose absorbed up to 24 hrs and that at ∞ , i.e., i.v. solution in monkey D: 129.57 vs 126.51%, oral in-line capsules in monkey D: 58.78 vs 60.36%. In other instances, however, these two percentages would differ greatly, i.e., oral solution in monkey B: 151.87 vs 216.83%, oral solution in monkey D: 99.70 vs 149.70%, P_1 in monkey D: 4.64 vs 6.45%. Table XIII shows that the difference between average percent of dose absorbed at 24 hrs and for oral solutions is much greater than the differences observed for in-line capsules and preparation G2.

Because of the discontinuous and unpredictable character of the enterohepatic recycling, very little can be said about the absolute bioavailability of indomethacin. Nevertheless, a relative estimate of the in vivo performance of the various formulations in the same monkey can be obtained by means of the ratio of total corrected AUC of the dosage form being evaluated to the total corrected AUC of the in-line preparation, (relative bioavailability). From the relative bioavailability value (11.07%) for preparation P1, shown in Table XIII, it is clear that this formulation did not release all of the dose given during the 8 hours of the experiment. This is also evidenced by Figs. 17 and 18. Even though no statistical inference is possible, the relative bioavailability estimates for the remainder of the controlled release preparations evaluated show that these formulations release the dose of the drug at about the same extent as the in-line capsules. Analysis of the plasma concentration versus time profiles provides data for the release patterns of drug from the dosage forms. For instance, Figs. 15 and 17 show that, in the same monkey, t_{max}

for G1 is exactly the same, 4 hours, as that for the in-line preparation; for G1, the subsequent plasma levels are slightly lower than those obtained from the in-line sample. The plasma concentration versus time profile provides evidence that G1 was not a satisfactory controlled release product, even though the total amount "absorbed" (71.5%) was higher than that observed after the administration of the standard sample (60.4%). Formulation P2, tested in Model II, showed an interesting plasma concentration-time profile (see Fig. 19): the plasma levels obtained during the first 6 hours post-administration were low; at the 8th hour, however, a plasma concentration of 4.1 mcg/ml was measured. This could be taken to indicate that, in fact, the dosage form delays and controls the rate of release of the drug. Figure 21 and Table XII show that by the 29th hour post-administration, 111.1% of the dose had entered the systemic circulation; even though this number represents drug absorbed and reabsorbed, it may be construed to indicate complete or almost complete bioavailability from the drug product. When preparation G2 was tested in monkey G, it showed a delayed release, t_{max}=6 hrs, and good plasma levels were observed between the 6th and 8th hrs as can be seen in Figs. 20 and 22. Preparation P3 in monkey G showed a t_{max}=3 hrs and good plateau from the 3rd to the 8th hr (see Figs. 19 and 21); from P3, 80.5% of the dose given had reached the systemic circulation by the 8th hour.

From the cumulative amount "absorbed" data, rate plots for some of the dosage forms studied were constructed as follows: the increment of amount absorbed in a sampling interval was divided by the time of the sampling interval giving an estimate of the mean rate of absorption;

these rate values were then plotted versus time at the mid-point of the sampling interval (t_{mid}). Figure 23 contrasts the "rate" plot for an oral solution (dose=100 mg) with that for the controlled release preparation P1 (dose=75 mg), both from studies in monkey D. From this plot it can be seen that while indomethacin was very rapidly absorbed from the solution, reaching the maximum rate (37.5 mg/hr) at 0.25 hr post administration and high rates were still observed for the remainder of the plotting time, drug from preparation P1 was rapidly absorbed (maximum rate=5.6 mg/hr) only within the first 0.75 hr and then the absorption decreased dramatically to values ranging from 0.1 to 0.35 mg/hr for the next 4 hours. For P1 the estimated rate of absorption at the 7th hour was 0.06 mg/hr. Figure 24, excretion rate versus t_{mid} on cartesian coordinate graph paper, shows the initial spike of rapid absorption and the subsequent zero order-like absorption observed after the administration of P1 to monkey D. This pattern could be explained in terms of an initial rapid release of indomethacin from the dosage form, followed by a very slow zero order-like release. Figure 25 is a combined graph showing the rates of absorption versus t_{mid}, on a semilogarithmic scale, obtained after the administration of the in-line, G2 and P3 preparations to monkey G (Model II). In this case it can be seen that in comparison with the in-line preparation, P3 produces initial delayed absorption and subsequently higher absorption rates of the drug at later times. A similar pattern was observed for preparation G2. However, in this case, the absorption was delayed even longer and the 5th to 7th hr absorption rates were higher than those for the in-line and P3 preparations. Figure 26,

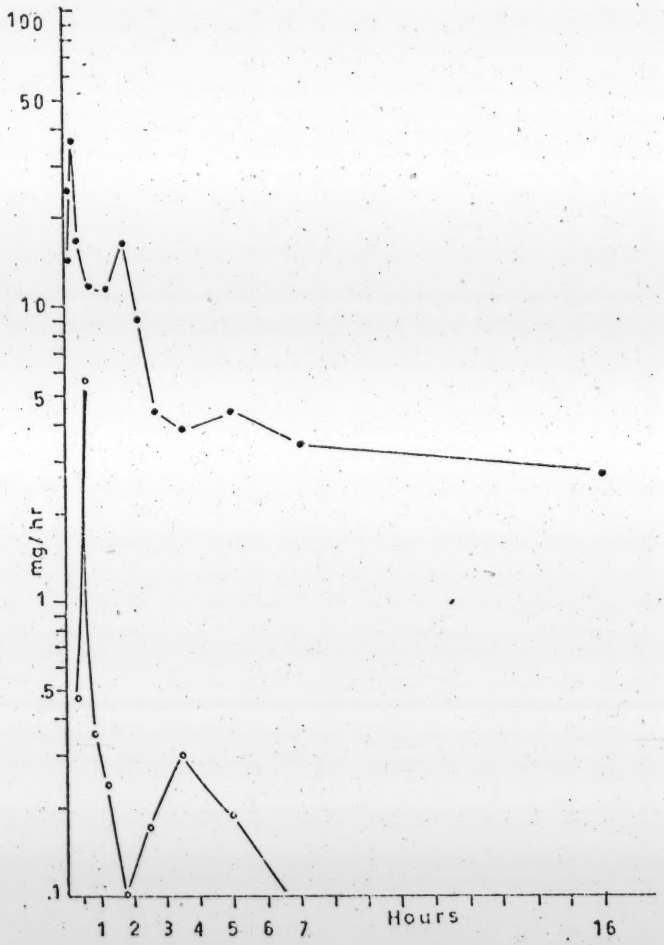


Fig. 23 - Semilogarithmic Excretion Rate versus t_{mid} Plot for: -●- an Oral Solution (100 mg) and -o- the Controlled Release Preparation P1 (75 mg) in Monkey D.

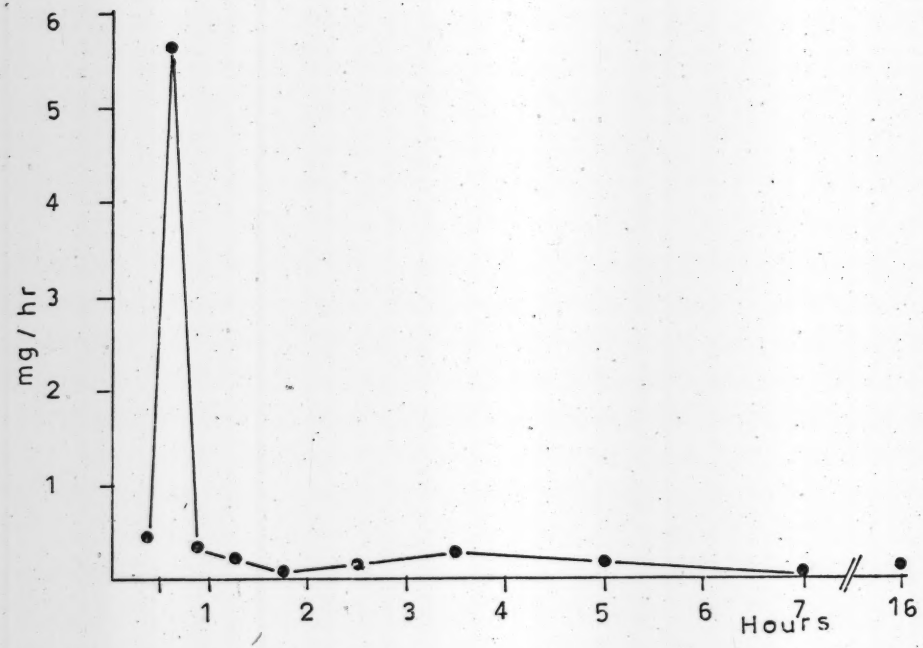


Fig. 24 - Excretion Rate (mg/hr) versus t_{mid} (hr) Profile after Oral Administration of Controlled Release Preparation P1 to Monkey D (Model I). Dose=75 mg.

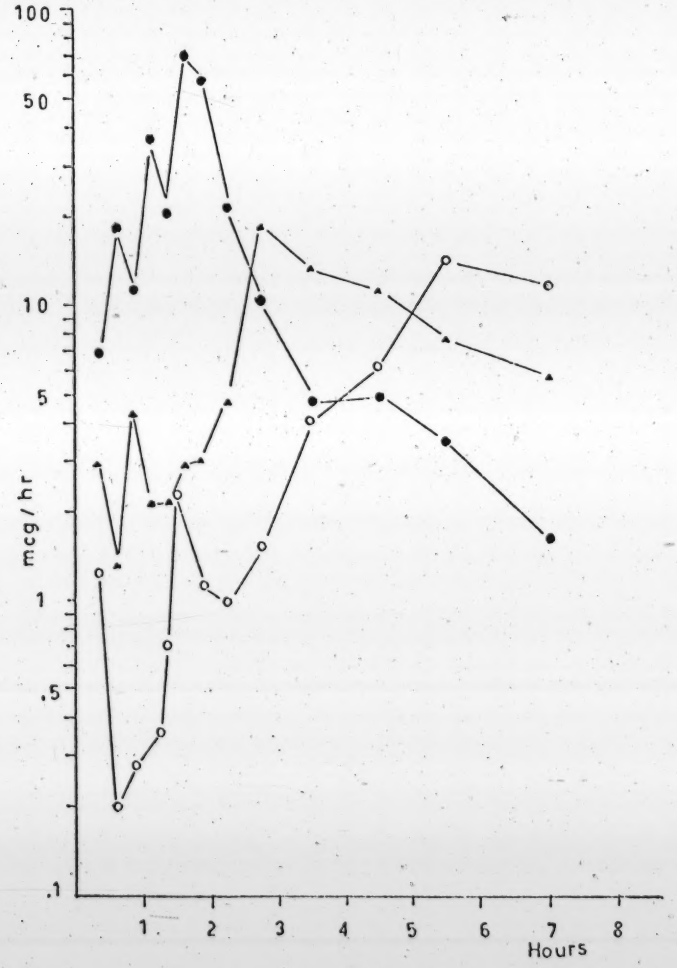


Fig. 25 - Semilogarithmic Excretion Rate versus t_{mid} Plot for: -●- In-line Preparation, -○- Controlled Release Preparation G2 and -Δ- Controlled Release Preparation P3 in Monkey G. Dose=75 mg.

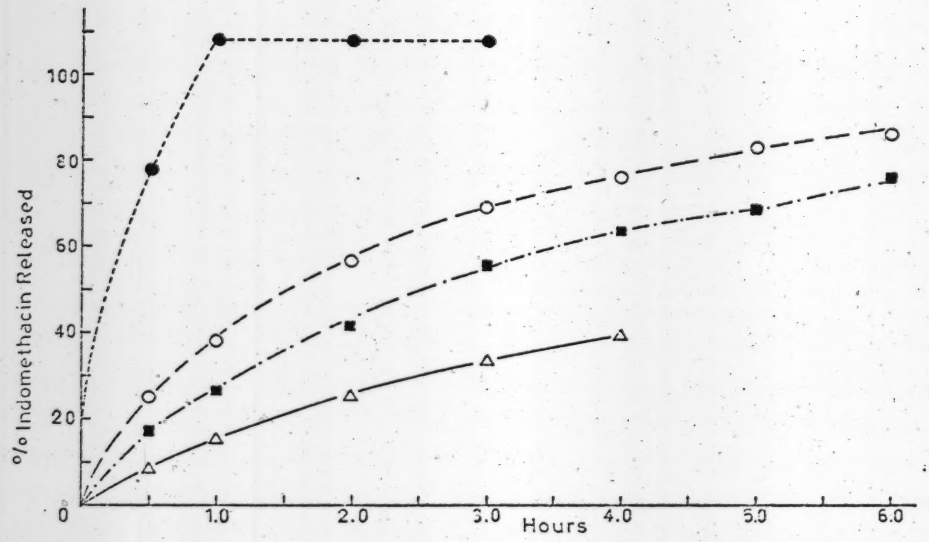


Fig. 26 - Percent of Indomethacin Released versus Time (Hr) Profile as Determined in Phosphate Buffer pH=6.2 from: ---●--- In-line Capsules, --○-- Controlled Release Preparation P3, -·-■-·- Controlled Release Preparation P2 and —△— Controlled Release Preparation G2.

obtained from the Merck, Sharp and Dohme Research Laboratories, shows the release of indomethacin from the in-line, P3, P2 and G2 preparations in phosphate buffer pH=6.2. By comparison of Figs. 26 and 25 a good rank order correlation is observed between the in vitro release of indomethacin and the in vivo absorption from these preparations as assessed in Model II. However, it should be noted that the in vivo release of P2 did not fall between that of P3 and G2 as found in vitro in phosphate buffer. In contrast in vivo release of drug from P2 was observed to be very much slower than the in vivo release for preparation G2.

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VI. SUMMARY AND CONCLUSIONS

Two alternative animal models for the evaluation of oral controlled release dosage forms under physiologically stabilized conditions have been developed. Unanesthetized restrained male rhesus monkeys with chronic vascular catheters and a plastic cannula surgically implanted in the stomach were utilized as the basic set-up. By radiological means, i.e., barium sulfate suspension and radiopaque beads, it was established that the intestinal transit time in fasted, unanesthetized restrained rhesus monkeys was considerably faster than the corresponding value in man. In order to make the gastrointestinal transit time in the monkey comparable to that in man, methods to modify and control transit time were considered. Two models were developed where passage to the large bowel was prevented and the contents of the small intestine were collected at the terminal ileum and readministered at the upper-jejunum. For Model I, surgical techniques were developed for the implantation of plastic cannulae in different portions of the small intestine of the animal: upper-jejunum and terminal ileum. For Model II, an ileostomy was performed, the colon permanently closed and a plastic cannula implanted in the upper-jejunum of the monkey.

Radiological studies were carried out to determine if the extensive surgery performed on Model I and Model II preparations had affected the gastrointestinal transit time. The post-surgery values found with either the barium sulfate suspension or the radiopaque beads for both models were not significantly different at the 0.05 significance level when compared to those obtained before surgery.

Model I and Model II allow the investigator to study the absorption

of drugs from controlled release preparations in a gastrointestinal system anatomically and physiologically similar to the g.i. system in humans. The models allow the direct administration of intact solid dosage forms into the stomach of the animal. The models allow the investigator to intravenously administer drug solutions and to sample the peripheral blood compartment frequently so that pharmacokinetic parameters of a drug can be determined. The models allow the controlled release dosage form to be in contact with the absorbing mucosa for a period of 8 to 12 hours. The models allow the investigator to run repeated studies on the same animal, for long periods of time during the course of each experiment in an unanesthetized state.

The models developed were tested utilizing experimental controlled release preparations of indomethacin (hard-gelatin capsules containing either coated granules of the drug, G1 and G2, or the drug embedded in a plastic polymer matrix, P1, P2 and P3).

Intravenous studies with indomethacin and oral studies with the drug in solution, in conventional (in-line) capsules and the controlled release preparations were conducted.

Pharmacokinetic parameters for indomethacin disposition in eight rhesus monkeys were obtained following the intravenous administration. Discontinuites in the plasma concentration - time curve apparently due to enterohepatic recycling of indomethacin in the monkey could be clearly seen. The total amount of drug reaching the systemic circulation after i.v. dosage was always greater than the administered dose.

The Loo-Riegelman method was used to estimate the cumulative amount of drug absorbed from plasma concentration time data obtained

after i.v. and oral administration of indomethacin to monkeys. In six monkeys, the extent of the enterohepatic recycling during the first 8 hrs post-administration averaged 31.1% (S.D.=20.4) and for up to 24 hours averaged 49.8% (S.D.=39.5%). The latter estimate agrees closely with the 50% reported in man.

Our studies show that indomethacin is rapidly absorbed when given orally as a solution. When indomethacin either in solution or in capsules was placed in the ileo-jejunal connection, the drug was rapidly absorbed from the terminal ileum and/or the upper jejunum.

Estimates of total area under the plasma concentration time curve corrected to the administered dose and body weight, percent of dose "absorbed" (Loo-Riegelman method), and relative bioavailability for the dosage forms under study were obtained. The enterohepatic recycling of indomethacin proved to be a complicating factor in the rigorous evaluation of the dosage forms under study. Nevertheless, a relative estimate of the *in vivo* performance of the various formulations in the same monkey was obtained by means of the ratio of total corrected AUC of the dosage form being evaluated to the total corrected AUC of the in-line preparation (relative bioavailability). Additionally, rate plots for some of the dosage forms under study were constructed from the cumulative amount of drug "absorbed" data.

Analysis of all data obtained lead to the conclusion that from the five experimental controlled release dosage forms of indomethacin (G1, G2, P1, P2, P3) tested, only preparations P2 and P3 (and perhaps G2) showed *in vitro* and *in vivo* availability characteristics that justify their further testing in human subjects.

APPENDIX

TABLE 1. COMPOSITION OF PURINA MONKEY CHOW.

PURINA MONKEY CHOW[R] 25

Guaranteed Analysis

Crude protein not less than	25.0%
Crude fat not less than	5.0%
Crude fiber not more than	3.5%
Added minerals not more than	3.0%
Ash not more than	5.0%

Ingredients:

Ground yellow corn, soybean meal, ground wheat, corn gluten meal, dried skimmed milk, animal fat preserved with BHA, sucrose, brewer's dried yeast, salt, dehydrated alfalfa meal, vitamin B_{12} supplement, riboflavin supplement, calcium pantothenate, niacin, choline chloride, menadione dimethyl pyrimidinol bisulfite (source of vitamin K activity), folic acid, pyridoxine hydrochloride, thiamin, ascorbic acid, vitamin A supplement, D activated animal sterol (source of vitamin D_3), vitamin E supplement, iron oxide, iron sulfate, manganese sulfate, calcium iodate, calcium carbonate, dicalcium phosphate, manganous oxide, copper oxide, cobalt carbonate, zinc oxide.

TABLE 2A. Sample Data Showing the Application of the Loo-Riegelman Method * for the Estimation of $C_{2_{tn}}$, After the I.V. Administration of Indomethacin to Monkey D, (Dose=27mg).

t_{tn}	$C_{1_{tn}}$	ΔC_1	Δt	$\frac{k_{12}}{2}\Delta C_1 \Delta t$	$C_{1_{tn-1}}$	$e^{-k_{21}\Delta t}$	$\frac{k_{12}}{k_{21}}(1-e^{-k_{21}\Delta t})$	$C_{1_{tn-1}} \cdot \frac{k_{12}}{k_{21}}(1-e^{-k_{21}\Delta t})$	$C_{2_{tn-1}}$	$C_{2_{tn-1}} \cdot e^{-k_{21}\Delta t}$	$C_{2_{tn}}$
0	126	-	-	-	-	-	-	-	-	-	-
0.083	59.5	-66.5	0.083	-1.901	126	0.944	0.056	7.002	-	-	5.101
0.167	29	-30.5	0.083	-0.872	59.5	0.944	0.056	3.332	5.101	4.185	7.275
0.333	15	-14	0.167	-0.805	29	0.891	0.108	3.151	7.275	6.482	8.828
0.5	2.2	-12.8	0.167	-0.736	15	0.891	0.108	1.620	8.828	7.866	8.750
1.0	0.8	-1.4	0.5	-0.241	2.2	0.707	0.291	0.640	8.750	6.186	6.585
1.5	0.34	-0.46	0.5	-0.079	0.8	0.707	0.291	0.233	6.585	4.656	4.810
2	0.28	-0.06	0.5	-0.010	0.34	0.707	0.291	0.099	4.810	3.400	3.489
3	0.34	0.06	1	0.021	0.28	0.500	0.497	0.139	3.489	1.745	1.905
4	0.37	0.03	1	0.010	0.34	0.500	0.497	0.169	1.905	0.952	1.131
6	0.24	-0.13	2	-0.090	0.37	0.250	0.746	0.276	1.131	0.283	0.469
8	0.08	-0.16	2	-0.110	0.24	0.250	0.746	0.179	0.469	0.117	0.186
24	0.03	-0.05	16	-0.276	0.08	$1.5\ 10^{-5}$	0.994	0.080	0.186	0.000	-0.196

* The Equation: $C_{2_{tn}} = C_{1_{tn-1}} \cdot \frac{k_{12}}{k_{21}}(1 - e^{-k_{21}\Delta t}) + C_{2_{tn-1}} \cdot e^{-k_{21}\Delta t} + \frac{k_{12}}{2} \cdot \Delta C_1 \cdot \Delta t$

TABLE 2B. Sample Data Showing the Application of the Loo-Riegelman Method* to Estimate the Amount of Drug Reaching the Systemic Circulation After I.V. Administration of Indomethacin to Monkey D, (Dose = 27 mg).

t_{tn}	$C_{1_{tn}}$	$C_{2_{tn}}$	$\int_0^{tn} C_1\,dt$	$k_{13}\int_0^{tn} C_1\,dt$	$(A_{tn})/V_1$	A_{tn}
0	126.0	-	-	-	126.0	26.99
0.083	59.5	5.101	7.698	59.326	123.927	26.55
0.167	29.0	7.275	11.415	87.972	124.247	26.62
0.333	15.0	8.828	15.067	116.117	139.945	29.98
0.5	2.2	8.750	16.503	127.184	138.134	29.60
1.0	0.80	6.585	17.253	132.964	140.349	30.07
1.5	0.34	4.810	17.538	135.160	140.310	30.06
2	0.28	3.489	17.693	136.355	140.124	30.02
3	0.34	1.905	18.003	138.744	140.990	30.21
4	0.37	1.131	18.358	141.480	142.981	30.64
6	0.24	0.469	18.968	146.181	146.890	31.47
8	0.08	0.186	19.288	148.568	148.834	31.89
24	0.03	-0.196	20.223	155.853	155.687	33.36

* The Equation: $(A_1)_{tn} / V_1 = C_{1_{tn}} + C_{2_{tn}} + k_{13}\int_0^{tn} C_1\,dt$.

TABLE 2C. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After the Oral Administration of Indomethacin in Solution to Monkey B, (Dose = 90 mg).

t_{tn}	$C_{1_{tn}}$	$C_{2_{tn}}$	$\int_0^{tn} C_1 dt$	$k_{13}\int_0^{tn} C_1 dt$	$(A_{tn})/V_1$	A_{tn}
0	0	0	0	0	0	0
0.25	7.5	0.92	0.938	6.159	14.579	5.85
0.5	17.5	3.750	4.063	26.678	47.928	19.23
0.75	6.7	5.986	7.08	46.540	59.226	23.76
1.0	3.35	6.380	8.344	54.787	64.517	25.885
1.25	2.8	6.310	9.113	59.836	68.946	27.66
1.5	3.2	6.238	9.863	64.760	74.198	29.77
2	3.15	6.193	11.450	75.181	84.524	33.91
2.5	3.3	6.189	13.063	85.772	95.261	38.22
3	3.15	6.165	14.675	96.356	105.671	42.40
4	2.1	5.576	17.300	113.592	121.268	48.65
5	2.25	5.003	19.475	127.873	135.126	54.21
6	2.5	4.831	21.850	143.467	150.798	60.50
7	4.3	5.678	25.250	105.792	175.770	70.52
8	3.9	6.474	29.350	192.712	203.086	81.48
10	0.9	4.144	34.150	224.229	229.273	91.99
12	1.5	3.171	36.550	239.987	224.658	98.16
14	1.25	2.723	39.300	258.044	262.017	105.12
18	2.65	5.148	47.100	309.259	317.057	127.21
20	1.0	3.460	50.750	333.225	337.685	135.48
24	1.85	3.762	56.450	370.651	376.263	150.96
27	0.75	1.954	60.350	396.258	398.962	160.07
30	0.43	-3.153	62.120	407.880	405.157	162.55
33	0.63	0.257	63.710	418.320	419.207	168.19
34	0.80	1.463	64.425	423.015	425.278	170.63
48	0.20	-2.579	71.425	468.977	466.598	187.21
72	0.01	-1.850	73.945	485.523	483.683	194.06

TABLE 2D. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin in Solution to Monkey C. (Dose = 100 mg).

t_{tn}	$C_{1_{tn}}$	$C_{2_{tn}}$	$\int_0^{tn} C_1 dt$	$k_{13} \int_0^{tn} C_1 dt$	$(A_{tn}) / V_1$	A_{tn}
0	0	0	0	0	0	0
0.083	7.5	0.220	0.311	0.832	8.552	6.94
0.167	26.0	1.175	1.718	4.597	31.772	25.79
0.333	44.0	3.112	7.528	20.145	67.257	54.60
0.5	27.5	6.674	13.499	36.123	70.297	57.07
0.75	15.0	8.924	18.811	50.338	74.262	60.29
1.0	11.0	9.537	22.061	59.035	79.572	64.60
2.0	6.9	8.774	31.011	82.985	98.659	80.09
2.5	5.2	7.890	34.036	91.080	104.170	84.57
3	3.9	6.837	36.311	97.168	107.905	87.60
4	3.5	5.176	40.011	107.069	115.745	93.96
6	1.95	4.912	45.461	121.650	128.512	104.33
8	1.45	2.310	48.861	130.750	134.510	109.20
24	1.4	1.152	71.661	191.760	194.312	157.74
28	1.25	1.300	77.161	206.480	209.130	169.77

TABLE 2E. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of In-Line Indomethacin Capsules to Monkey D, (Dose 100=mg).

t_{tn}	C_{1t_n}	C_{2t_n}	$\int_0^{t_n} C_1 dt$	$k_{13}\int_0^{t_n} C_1 dt$	$(A_{1t_n}) / V_1$	$A_1 t_n$
0	0	0	0	0	0	0
0.33	0	0	0	0	0	0
0.5	0.03	0.002	0.003	0.200	0.052	0.01
0.75	0.06	0.011	0.014	0.106	0.177	0.04
1.	0.10	0.025	0.034	0.261	0.385	0.08
1.5	0.05	0.038	0.071	0.550	0.638	0.14
2.	0.08	0.047	0.104	0.800	0.927	0.20
2.5	1.10	0.240	0.399	3.073	4.413	0.95
3.	2.5	0.733	1.299	10.009	13.242	2.84
4.	12.0	4.880	8.549	65.882	82.762	17.73
6.	1.4	2.860	21.949	169.151	173.410	37.15
8.	1.4	1.760	24.729	190.575	193.735	41.51
24.	0.05	-5.830	36.349	280.126	274.350	58.78

TABLE 2F. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of In-line Indomethacin Capsules to Monkey G, (Dose = 75 mg).

t_{tn}	$C_{1_{tn}}$	C_{2tn}	$\int_0^{tn} C_1 dt$	$k_{15}\int_0^{tn} C_1 dt$	$(A_{1_{tn}})/V_1$	A_{1tn}
0	0	0	0	0	0	0
0.083	0	0	0	0	0	0
0.25	0.2	0.016	0.017	0.068	0.284	0.13
0.5	2.5	0.324	0.354	1.438	4.262	1.87
0.75	7.1	1.369	1.554	6.310	14.779	6.50
1.0	5.8	2.628	3.167	12.857	21.285	9.35
1.25	14.6	4.637	5.717	23.210	42.447	18.66
1.5	11.2	6.947	8.942	36.303	54.450	23.93
1.75	28.3	10.640	13.880	56.353	95.293	41.88
2.	27.8	15.647	20.892	84.822	128.269	56.37
2.5	9.9	19.881	30.317	123.087	152.868	67.19
3.	6.2	19.215	34.342	139.429	164.844	72.45
4.	2.8	15.312	38.842	157.699	175.811	77.27
5.	3.5	12.183	41.992	170.488	186.171	81.82
6.	2.6	9.968	45.042	182.871	195.439	85.90
8.	0.8	5.569	48.442	196.675	203.044	89.24

TABLE 2G. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin Controlled Release Preparation P1 to Monkey D, (Dose=75 mg).

t_{tn}	C_{1tn}	C_{2tn}	$\int_0^{tn} C_1\,dt$	$k_{13}\int_0^{tn} C_1\,dt$	$(A_{1tn})/V_1$	A_{1tn}
0.	0.	0.	0.	0.	0.	0.
0.25	0.15	0.013	0.019	0.145	0.308	0.07
0.5	0.27	0.045	0.071	0.550	0.865	0.19
0.75	0.16	0.088	0.125	0.963	1.21	0.26
1.	0.22	0.105	0.173	1.330	1.65	0.35
1.5	0.09	0.161	0.250	1.930	2.18	0.47
2.	0.07	0.143	0.290	2.23	2.45	0.52
3.	0.12	0.124	0.385	2.97	3.21	0.69
4.	0.22	0.156	0.555	4.28	4.65	0.99
6.	0	0.355	0.775	5.97	6.33	1.36
8.	0.09	0.151	0.865	6.67	6.91	1.48
24.	0.06	0.255	2.065	15.91	16.23	3.48

TABLE 2H. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin Controlled Release Preparation G1 to Monkey D, (Dose= 75 mg).

t_{1_n}	$C_{1_{t_n}}$	$C_{2_{t_n}}$	$\int_0^{t_n} C_1\,dt$	$k_{13}\int_0^{t_n} C_1\,dt$	$(A_1)_{t_n}/V_1$	$A_{1_{t_n}}$
0.	0.	0.	0.	0.	0.	0.
0.5	0.	0.	0.	0.	0.	0.
1.	0.15	0.026	0.075	0.578	0.754	0.16
1.5	1.05	0.217	0.375	2.890	4.157	0.89
2.	5.6	-0.325	2.038	15.702	20.98	4.50
2.5	6.6	1.573	5.088	39.207	47.38	10.15
3.	6.0	2.930	8.238	63.483	72.413	15.50
4.	12.7	6.755	17.588	135.549	155.00	33.20
5.	1.6	5.866	24.738	190.642	198.11	42.44
6.	0.75	3.435	25.913	199.697	203.89	43.68
8.	0.12	0.984	26.783	206.402	207.50	44.46
24.	0.10	0.009	28.543	219.966	220.08	47.15

TABIE 2I. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin Controlled Release Preparation P2 to Monkey G, (Dose Equiv.= 75 mg).

t_{tn}	$C_{1\,tn}$	$C_{2\,tn}$	$\int_0^{tn} C_1\,dt$	$k_{13}\int_0^{tn} C_1\,dt$	$(A_{1\,tn})/V_1$	$A_{1\,tn}$
0	0	0	0	0	0	0
0.083	0	0	0	0	0	0
0.25	0.5	0.039	0.042	0.171	0.710	0.31
0.5	0.44	0.137	0.159	0.646	1.223	0.54
0.75	0.19	0.190	0.238	0.966	1.346	0.59
1.0	0.31	0.225	0.301	1.222	1.757	0.77
1.25	0.31	0.269	0.378	1.535	2.114	0.93
1.5	0.44	0.324	0.472	1.916	2.680	1.18
1.75	0.19	0.357	0.551	2.237	2.784	1.22
2.0	0.31	0.374	0.613	2.489	3.173	1.39
2.5	0.5	0.472	0.816	3.313	4.285	1.88
3	0.44	0.570	1.051	4.267	5.277	2.32
4	0.35	0.650	1.446	5.871	6.871	3.02
5	0.31	0.658	1.776	7.211	8.179	3.59
6	0.25	0.623	2.056	8.347	9.220	4.05
8	4.1	4.124	6.406	26.008	34.232	15.05
29	0.38	-27.720	53.446	216.991	189. 651	83.35

TABLE 2J. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin Controlled Release Preparation P3 to Monkey G, (Dose Equiv.=75 mg).

t_{tn}	$C_{1_{tn}}$	$C_{2_{tn}}$	$\int_0^{tn} C_1 dt$	$k_{13}\int_0^{tn} C_1 dt$	$(A_{1_{tn}}) / V_1$	$A_{T_{tn}}$
0	0	0	0	0	0	0
0.083	0	0	0	0	0	0
0.25	0.25	0.019	0.021	0.085	0.354	0.16
0.5	1.1	0.170	0.190	0.771	2.041	0.90
0.75	0.74	0.351	0.420	1.705	2.796	1.23
1.0	1.75	0.593	0.731	2.968	5.311	2.33
1.25	1.2	0.849	1.100	4.466	6.515	2.86
1.5	1.1	1.010	1.387	5.631	7.741	3.40
1.75	1.35	1.173	1.694	6.878	9.401	4.13
2.0	1.5	1.361	2.050	8.323	11.184	4.92
2.5	2.4	1.918	3.025	12.282	16.600	7.30
3.	2.0	4.055	5.875	23.853	36.908	16.22
4.	5.7	7.772	13.225	53.694	67.166	29.52
5.	6.2	9.443	19.175	77.851	93.494	41.09
6.	3.75	9.516	24.150	98.049	111.315	48.92
8.	3.2	7.947	31.100	126.266	137.413	60.39

TABLE 2K. Cumulative Amount of Drug Reaching the Systemic Circulation (Loo-Riegelman Method), After Oral Administration of Indomethacin Controlled Release Preparation G2 to Monkey G, (Dose=75 mg).

t_{tn}	$C_{1_{tn}}$	$C_{2_{tn}}$	$\int_o^{tn} C_1 dt$	$k_{15} \int_o^{tn} C_1 dt$	$(A_{1_{tn}})/V_1$	$A_{1_{tn}}$
0	0	0	0	0	0	0
0.083	0	0	0	0	0	0
0.25	0.2	0.023	0.017	0.069	0.292	0.13
0.5	0.5	0.099	0.104	0.422	1.021	0.45
0.75	0.20	0.164	0.192	0.780	1.144	0.50
1.0	0.15	0.184	0.236	0.958	1.292	0.57
1.25	0.17	0.200	0.276	1.121	1.491	0.66
1.5	0.30	0.231	0.334	1.356	1.887	0.83
1.75	0.90	0.341	0.484	1.965	3.206	1.41
2.	0.65	0.473	0.678	2.753	3.876	1.70
2.5	0.50	0.613	0.966	3.924	5.037	2.21
3.	0.80	0.766	1.291	5.241	6.807	2.99
4.	1.8	1.625	2.591	10.519	13.944	6.13
5.	3.65	3.236	5.316	21.583	28.469	12.51
6.	7.5	6.57	10.891	44.217	58.287	25.62
8.	6.05	10.496	24.441	99.230	115.776	50.88